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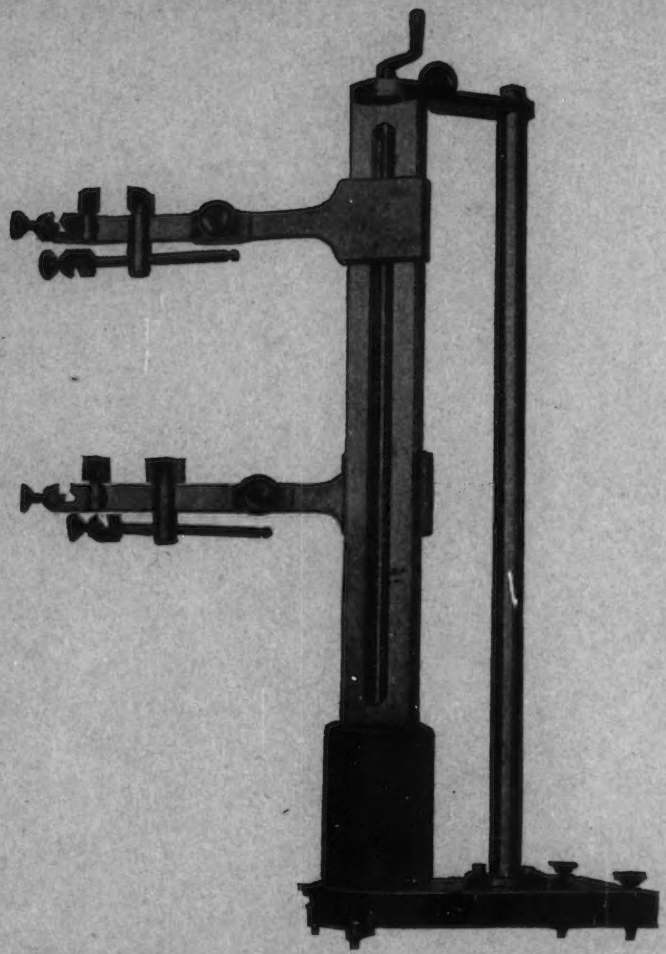

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THE EQUATION EXPRESSING THE EXCRETION OF A DIURETIC AND ITS RELATION TO DIFFUSION PROCESSES

PART II

E. J. CONWAY

From the Department of Physiology, University College, Dublin

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I. In a previous communication (Conway and Kane, 1926) formulae experimentally determined for renal secretion were explained in accordance with diffusion laws and a diffusion-secretion theory put forward.

This theory in outline states that the total volume of urine comes from the glomeruli, being similar in composition to a blood filtrate and that all substances appearing in the urine in greater concentration than in the blood are added by diffusion from the tubule cells. Diffusion as in this case to a higher concentration may be exemplified in vitro by the diffusion of iodine from a stream of water into chloroform. This, it was shown, can be expressed by the relation

$$\sqrt{\frac{1}{t}}\,\frac{C_t - C_i}{PC_o - C_i} = K_1 \qquad 1$$

Here C_t = total concentration in the chloroform after time t; C_i = concentration in the chloroform at zero time; C_o = concentration of iodine in water layer; and P is the partition coefficient. $PC_o - C_i$ may be described as the "diffusion head" of the iodine.

The equation may be written

Substance diffused in time t = $\sqrt{t}$ × the diffusion head × a constant.

This has the same form as that mathematically deduced for simple diffusion into a cylinder and all the equations in this communication relating to secretion or diffusion may be considered merely as different statements or

special cases of this fundamental relation to which the title of the paper may be taken to refer.

If the diffusion head were fixed in (1), the equation might be written

$$\sqrt{\frac{1}{t}}\,(C_t - C_i) = K_2 \qquad 2$$

which has the same form as the excretion equation

$$\sqrt{\frac{1}{t}}\,(C_u - C_B) = K_3 \qquad 3$$

(Conway, 1925; Conway and Kane, 1926) where t = time of unit volume of urine, C_u = concentration of urine and C_B = concentration of plasma. This equation was shown to apply empirically at high values of C_B to glucose, urea, and with a modification to sodium chloride. Here the diffusion head would be $(C_{max} - C_B)$, the equation being more fully written

$$\sqrt{\frac{1}{t}}\,\frac{C_u - C_B}{C_{max} - C_B} = K_4 \qquad 4$$

where C_{max} may be taken as the upper limit of C_u as the urine rate is indefinitely decreased, the secretion conditions being otherwise constant. The diffusion head is regarded as constant beyond a certain plasma concentration which is taken provisionally at 0.0375M.

This differs slightly from the previous treatment of the question where C_{max} under similar conditions was regarded as constant, $(C_{max} - C_B)$ being so only by implication, as C_B is always small compared to C_{max}.

In the equation 1, for iodine diffusion, if C_i is negligible compared to C_t we have (from 1)

$$\sqrt{\frac{1}{t}}\,\frac{C_t}{PC_o} = K_5 \qquad 5$$

which is analogous to the secretion equation for urea in the human subject (Austin, Stillman and Van Slyke, 1921). This at constant body weight reduces to

$$\sqrt{\frac{1}{t}}\,\frac{C_u}{C_B} = K_6 \qquad 6$$

The diffusion head may be again taken as $(C_{max} - C_B)$ or C_{max}, since C_B is very small in comparison, and at these low "normal" plasma concentrations is proportional to C_B, i.e., $C_{max} = P_1 \times C_B$ where P_1 is a constant similar to the partition coefficient in equation 5.

The secretion equations 3 and 6 apply theoretically only within the limit of the energy that the kidney can utilize. The possibilities of such a limit are considered later (section V) where reasons are given for the diffusion formula then becoming.

$$\frac{\text{Output of urea}}{\text{Blood concentration}} \text{ or } \frac{V\,C_u}{C_B} = \text{a constant} \qquad 7$$

an equation established experimentally by Austin, Stillman and Van Slyke (1921) and by Addis and Drury (1923a, 1923c) beyond a certain value of V.

The theory so far stated does not include those instances where the urinary concentration of an excreted substance such as sodium chloride is less than in the plasma. This is dealt with in a subsequent communication.

It may be pointed out that there must be a lower limit of $\frac{1}{t}$ or V in the equations (1) to (6) since in equation 4, for example, $\frac{C_u - C_B}{C_{\max} - C_B}$ is necessarily limited to unity.

The limit of application may be taken from the theoretical formulation of the complete diffusion equation (Carslaw, 1921) at a ratio

$$\frac{C_u - C_B}{C_{\max} - C_B} = 0.75$$

At higher values than this the value of the constant rapidly falls. It will appear from this that when the secretion formula (3) has reached its lower limit of V (or $\frac{1}{t}$) the value of $(C_u - C_B)$ can show a further increase of approximately 25 per cent as the value of V approaches zero. The average maximum urinary concentrations theoretically attainable at high blood values would thus be higher than those considered as such in a previous paper (Conway, 1925). Evidence of such a slow progressive rise in concentration of glucose was obtained below this volume limit for application of the formula. The increase corresponds to comparative figures obtained from the generalized diffusion equation. Taking this consideration into account the diffusion head for urea corrected for 39°C. at high blood values is 0.77M, for glucose, 0.90M and for sodium chloride 0.153 (expressed in mols of NaCl per litre).

Addis and Drury (1923c) have published results on 18 normal rabbits in which the average urine urea was 0.94M, with an average urine volume of 0.028 cc. per minute per kidney. At this volume diffusion may be regarded as complete so that the figure for C_u would represent the true maximum in these circumstances. The "diffusion head" would thus be

approximately 0.90M, the same as that calculated for glucose. The difference between the two urea figures for the "diffusion head" obtained from the data of Addis and Drury (1923c) and from Conway and Kane (1926), may be due to some interference with chloride, as the urea in the experiments of the latter observers was injected in 0.6 per cent saline. This is borne out by the fact that in Drury's experiments (1923), where urea dissolved in 0.9 per cent saline was injected into rabbits the value of $\sqrt{\frac{1}{t}}(C_u - C_B)$ is only 4 per cent less than in the experiments of Conway and Kane (at 39°C.) thus indicating the similarity of the "diffusion head" $(C_{max} - C_B)$ in these conditions (see equation 4). The interference would make no difference to the application of the diffusion equations provided it was the same at the high and low urine volumes. It is likely then that 0.90M is approximately the average maximum diffusion "head" for the rabbit, other substances appearing in more than minute quantities causing a depression in this value for the chief constituent such as urea or glucose. (Conway and Kane, 1926; Conway, 1923.)

The facts relating to such interference are in general accordance with the observations of Chaussin (1920), concerning the "antagonism" between the concentrations of chlorides and urea (quoted and confirmed by Adolph, 1923); of Davies, Haldane, and Peskett (1922) concerning the quantitative interference of chlorides and bicarbonates and with the general conclusions of Adolph (1923). The nature of such interference is here viewed from a different standpoint, and it may be noted that according to the present theory, none should occur as a rule until the sum of the plasma concentrations produces the maximum diffusion head.

The diffusion secretion theory involves in addition to the established facts of diffusion, the applicability of the diffusion laws to diffusion into fluids flowing through capillary tubes. This is treated in section II. It is also desirable to extend observations based on urea, sodium chloride and glucose to other substances, which is considered in section III. Further, the theory involves that the secretion of various substances is governed by their diffusion coefficients. The manner in which these enter into equation 4, appears in the following

$$\sqrt{\frac{1}{t \times D}} \times \frac{C_u - C_B}{C_{max} - C_B} = K_s \qquad 8$$

This constant K_s, at high blood values becomes then a general constant for secretion by the same length of tubule. Evidence connected with this relation appears also in section III.

Various other formulae have been proposed by different observers for the

secretion of urea particularly in the human subject (e.g., Ambard 1910a, 1910b; Walker and Rowe 1927b).

The grounds for these formulae are critically examined and it is shown that the diffusion-secretion formulae is the only one strictly applicable.

II. The formula

$$\sqrt{\frac{1}{t}}\frac{C_t}{C_o} = K, \qquad 9$$

can be deduced from Fourier calculations based on Fick's diffusion law. It has been shown that this formula applies to diffusion of the kind represented by the passage of iodine from a stream of iodine water into chloroform (Conway and Kane, 1926).

TABLE 1

TUBE	V (PERFUSION RATE)	C_t (PER CENT CONCENTRATION OF GLUCOSE IN COLLECTED FLUID)	$\sqrt{V}\,C_t$
	cc. per minute		
I	0.407	0.316	0.202
II	0.224	0.481	0.228
III	0.227	0.659	0.314
IV	0.251	0.490	0.245
Average.....	0.277		0.247
V	2.622	0.123	0.199
VI	5.168	0.127	0.289
VII	3.454	0.096	0.178
VIII	5.605	0.110	0.260
IX	7.638	0.088	0.243
Average.....	4.897		0.234

Evidence will now be given for the applicability of the formula to fluid passing through a capillary tube where the concentration of solute in the walls is constant and stream line motion is occurring. For the purpose solid cylinders of clear uncrystallized sugar have been used. These were made from a mixture of cane sugar and glucose in the proportion of about 5 to 1 (the glucose prevents crystallization), with the addition of sufficient water to bring the boiling point of the mixture to 129°. Through these cylinders passed a fine steel wire one-hundredth of an inch in diameter. Through the capillary tube formed on withdrawing the wire, water was perfused at varying rates for each tube. Contact of fluid with ends of tube outside capillary was prevented by paraffin wax, applied before with-

drawing the wire. On perfusing, the bore of the tube rapidly enlarges. This would have no effect theoretically on the application of the diffusion equations if the enlargement were uniform. This however is not the case, more sugar being removed at the beginning than at the end of the tube owing to the change in the diffusion gradient. To obviate this as much as possible the fluid was collected only at the beginning of the perfusion, and a different tube of the same composition used for each observation. The nearer the observation to the beginning of the perfusion the closer became the applicability of the formula.

The collected fluid was analysed for glucose, and in some cases for cane sugar also. Table 1 gives the results from 9 tubes, where the perfusion was analyzed for glucose. The samples were taken within about 5 minutes from the beginning of perfusion. Four of the tubes were taken at low

TABLE 2

TUBE	MID-TIME OF COLLECTION FROM $t = 0$	$\sqrt{\frac{1}{t}}\,C_t$ (GLUCOSE)	$\sqrt{\frac{1}{t}}\,C_t$ (CANE SUGAR)	V or $\frac{1}{t}$
	minutes			
I	1.25	0.289		5.168
II	2.00	0.245	1.42	0.251
III	2.62	0.260	1.47	5.605
IV	2.75	0.243	1.35	7.368
V	5.00	0.314	1.62	0.227

perfusion rates and 5 at high. It will be seen that an average increase of 16.7 times in the perfusion rate causes an average decrease of only 5 per cent in the value of $\sqrt{\frac{1}{t}}\,C_t$, where t is the time of unit volume, and C_t the concentration in the collected fluid. The variability of the individual results can be attributed to varying degrees of uniformity in bore. Table 2 gives the results of 5 tubes where particular attention was paid to the time of collection in its relation to the beginning of the perfusion. The highest figure in the series for $\sqrt{\frac{1}{t}}\,C_t$ was obtained after the longest previous perfusion. The results show that the diffusion equation 9 applies to diffusion under the conditions stated. It may be taken that if the perfusion fluid had an initial concentration C_i, then the formula

$$\sqrt{\frac{1}{t}}\,(C_t - C_i) = K_2$$

would apply and if tubes yielding different wall concentrations were chosen then we should have

$$\sqrt{\frac{1}{t}}\frac{C_t}{C_o} = K_9$$

where C_o is the concentration in the walls. It may be objected to these experiments that the bore is changing and that the change is not uniform. To these objections it may be replied (without laying too much stress on the experiments in question) that it can be shown theoretically for simple diffusion into a cylinder that the relation $\sqrt{\frac{1}{t}}\frac{C_t}{C_o}$ is independent of the bore; also that for at least the first five minutes of the perfusion here described, when the change in bore is most rapid, it has no effect upon the given relations (see table 2). The fact that owing to the diffusion process more sugar is removed at the beginning of the tube than towards the end would make it appear desirable to obtain evidence in relation to a tube of unchanging bore. Such evidence is difficult to obtain in regard to a fine capillary tube, as if any ordinary membrane be used the pressure necessary to drive the fluid through the capillary causes a leak through the walls. The present experiments curiously enough would seem to be actually more pertinent to the tubule conditions since here owing to the pressure gradient necessary to expel the urine, the lumen at the beginning of the secreting region will probably be more distended. Considering then that the theoretical diffusion formulae for simple diffusion apply to diffusion across two phases where a partition coefficient is operative (Conway and Kane, 1926) and also to diffusion into a capillary of the kind here described, their consideration in relation to the kidney tubule is justified.

III. The diffusion equation at high blood values. It has been previously shown that equation 3 applies to the secretion of glucose and urea at high blood concentrations and to sodium chloride (with a modification) after large injections. It will now be shown from the figures of Mayrs, that it applies also to sulphate when the blood concentration is much increased by injections of sodium sulphate, and further that the relation

$$\Sigma \sqrt{\frac{V}{D}}(C_u - C_B) = K_{10} \qquad 10$$

holds when sulphate is injected with urea, phosphate, etc. Here K_{10} is the same constant as that obtained after sulphate alone has been injected, D being the diffusion coefficient of the injected substance.

Mayrs in three papers (1922, 1923) gives figures for the excretion of

sulphate where the blood concentrations are high following injections. The body temperatures are not given, and one of the papers (Mayrs and Watt, 1922) is not quite suitable for the present discussion as the conditions were favourable to a fall of temperature unless special precautions were taken to prevent it. In the other papers, 24 experiments are recorded, 23 of which show blood values beyond 0.03–0.04M, i.e., suitable for applying the equation. Five of the experiments are recorded after sulphate injections only, four after sulphate and creatinine, four after sulphate and phosphate and ten after sulphate and urea. As the creatinine concentrations are comparatively very small, one could regard the sulphate and creatinine experiments as those after purely sulphate injections, giving altogether nine such experiments, which are summarized in table 3.

In dealing with the other figures of mixed injections we must take account of the diffusion coefficients and of ionization and consider the excretion

TABLE 3

AVERAGE URINE VOLUME	AVERAGE VALUE OF $\sqrt{V}\ (C_u - C_B)$	AVERAGE VALUE OF OUTPUT (GRAMS PER HOUR) / PLASMA CONCENTRATION	AVERAGE BODY WEIGHT	NUMBER OF EXPERIMENTS
cc. per minute per kidney			*kgm.*	
2.56	1.36	13.3	2.37	3
1.09	1.30	5.5	2.14	3
0.52	1.35	3.3	1.73	3

In the table C_u (urine concentration) and C_B (plasma concentration) are expressed as grams per 100 cc. In the second column V is taken as the urine volume per 2 kilos body weight.

from the standpoint of molecular units. The ionization in question is that at which sulphate or phosphate is excreted, i.e., in the urine film adjacent to the cells. If we take the concentrations in this film as that of the urine concentration at $t = \infty$ then the molecular concentration of the sulphate radicle in the urine must be multiplied by 1.92 (as determined from the figures for the electrical conductivity, Mellor, 1922). There is a rather wide latitude however, in this matter of ionization, as at 6.9 per cent the factor is 1.92, at 5.0 per cent 1.99 and at 4.0 per cent, 2.03. This total ionization factor of sulphate differs but slightly from that for phosphate since the determinations of the freezing points of neutral sodium phosphate in equivalent solutions show only about a 3 per cent difference (an allowance to this extent has been made in dealing with the phosphate excretion). In regard to the diffusion coefficients, the comparative values of D for sulphate and urea are 0.66 (Mellor, 1922) and 0.97 (Heimbrodt, 1904). Neutral sodium phosphate it is likely may be taken as closely similar to sulphate. For creatinine a diffusion coefficient of 0.73

has been used from comparison of the molecular weights, of creatinine and urea. However owing to the small part played by creatinine in these experiments, an inexactitude is without significance. Taking the sulphate diffusion coefficient as unity it will be necessary to multiply the urea value of $\sqrt{V}\ (C_u - C_B)$ by $0.82 \left(= \sqrt{\frac{0.66}{0.97}} \right)$ when adding to it the value for sulphate, a similar procedure being adopted with the other substances.

The result appears from Mayrs' figures that the total value of $\sqrt{\frac{V}{D}}\ (C_u - C_B)$ may be regarded as constant. The results are summarized in

TABLE 4

INJECTION	$\sqrt{\frac{V}{D}}\ (C_u - C_B)\ D$ FOR SULPHATE = 1						$\Sigma \sqrt{\frac{V}{D}}\ (C_u - C_B)$ EXPRESSED AS RATIO OF TOTAL AVERAGE
	Sulphate	Urea	Creatinine	Phosphate	Total	Number of experiments	
Sulphate	0.178	0.008			0.186	5	0.98
Sulphate + creatinine	0.184		0.0075		0.191	4	1.01
Sulphate + urea	0.131	0.048			0.179	10	0.94
Sulphate + phosphate	0.093			0.128	0.221	4	1.16

V = urine volume in cubic centimeter's per minute per kidney, for a body weight of 2 kilos. C_u and C_B are molecular concentrations in urine and plasma, allowance being made for ionization.

TABLE 5

INJECTION	AVERAGE K_{10} FOR OTHER SUBSTANCE / AVERAGE K_{10} FOR SULPHATE	AVERAGE C_B FOR OTHER SUBSTANCE / AVERAGE C_B FOR SULPHATE	NUMBER OF EXPERIMENTS
Sulphate + creatinine	0.041	0.035	4
Sulphate + urea	0.369	0.442	10
Sulphate + phosphate	1.376	1.225	4

table 4. The average value of $\Sigma \sqrt{\frac{V}{D}}\ (C_u - C_B)$ where sulphate alone was injected is 0.186. (See units under table 4.) This includes the value of $\sqrt{\frac{V}{D}}(C_u - C_B)$ for urea, though none was injected. The addition amounts to only 4 per cent of the sulphate value and was made for the reason that the object of the calculation is to show that the total excretion as represented by the diffusion equation remains constant. It is practically

certain that after large sulphate injections alone no other substance but urea will enter appreciably into the total excretion. The average value of $\Sigma \sqrt{\frac{V}{D}} (C_u - C_B)$ for all experiments after the injection of sulphate plus some other substance is 0.191. This may be about 2 per cent less than the true value since for sulphate plus creatinine, and sulphate plus phosphate injections, no urea analyses were given. Table 5 shows for the

TABLES 6 and 7

VOLUME RANGE	AVERAGE VOLUME	$K_{10} = \Sigma \sqrt{\frac{V}{D}} (C_u - C_B)$ (D FOR SULPHATE TAKEN AS UNITY)	K_{10} AS PER CENT OF TOTAL AVERAGE	NUMBER OF EXPERIMENTS
cc. per minute per kidney				
0.1–0.6	0.44	0.194	99	7
0.6–1.1	0.89	0.201	103	7
1.1–1.6	1.26	0.189	97	5
1.6–2.1				0
2.1–2.6	2.40	0.198	102	1
2.6–3.6				0
3.6–4.1	4.00	0.194	99	2
RANGE OF C_B (MOLECULAR CONCENTRATION CORRECTED FOR IONIZATION)	AVERAGE C_B			
0.05–0.09	0.07	0.203	104	10
0.09–0.13	0.10	0.172	88	5
0.13–0.17	0.14	0.207	106	5
0.17–0.21	0.19	0.185	95	2

experiments where two substances were injected that the ratio of the $\sqrt{\frac{V}{D}} (C_u - C_B)$ values tends to the same figure as the ratio of the plasma concentrations of the two substances. It seems however that for urea, although this is true if the normal blood concentrations be raised by injections, the sulphate excretion is more favoured than the urea if sulphate alone is injected and the blood urea remains comparatively very low (Mayrs, 1922). In general it appears from Mayrs' figures that when sulphate and some other substance are injected, the secretion of each as judged by the expression $\sqrt{\frac{V}{D}} (C_u - C_B)$ is proportional to the blood values.

The total secretory activity as judged by the value of K_{10} being constant

after mixed sulphate injections, it will now be shown that K_{10} is independent of the urine volume and the plasma concentration.

The value of K_{10} for Mayrs' 23 rabbits, omitting one aberrant result is 0.195 ± 0.023. The aberrant value is 0.084 and was obtained where the plasma sulphate had reached its highest value in the series, being 1.052 per cent Na_2SO_4 (anhyd.), the plasma urea at the same time being 0.308 per cent. Here it would seem that the kidney was failing through some effect of the very high blood concentration. Omitting this obviously aberrant result tables 6 and 7 illustrate the effect of volume and C_B on the constant K_{10} for 22 rabbits.

We may summarize the results by means of a statistical equation in which the very small effect of V or C_B on K_{10} is expressed as a linear equation of the form

$$K_{10} = a\,V + b \qquad 11$$

a and b being obtained so that $\Sigma\{K_{10} - (aV + b)\}^2$ is a minimum (Yule, 1924). The equation so deduced is

$$K_{10} = -\,0.0034\,V + 0.200 \qquad 12$$

The equation shows that a change in urine volume from 0.1 cc. per minute per kidney to 2.0 cc., i.e., a range of twenty times, decreases K_{10} only three per cent, this being within the error limits for the figures used. A similar equation for the whole of Mayrs' 34 rabbits receiving sulphate injections (1922a, 1922b, 1923) and for which the necessary data are given shows an increase of 6 per cent for the same range.

The effect of plasma concentration on K_{10} may be shown in the same way. The equation

$$K_{10} = -\,0.072\,C_B + 0.187 \qquad 13$$

gives the effect for the whole 34 rabbits. C_B is here the total plasma concentration of the injected substances, allowance being made for ionization. (All the urine volumes with these rabbits exceeded 0.1 cc. per minute and 33 of the 34 exceeded 0.0375 for the plasma value, one figure being 0.023 the corresponding value of K_{10} being close to the mean so that no difference would result from its exclusion). It will be seen from equation 13 that an increase of plasma concentration from 0.04 to 0.20, causes a decrease of only 6 per cent in K_{10}. A still smaller effect results if we confine ourselves to the two papers where the conditions in regard to body temperature are more likely to have been maintained.

We may conclude that the value of K_{10} after sulphate or mixed sulphate injections is independent of urine volume and plasma concentration. Another opportunity for the application of the diffusion equation for high

blood values to the work of independent observers is given by the results of Drury with urea (1923). This is of the more importance as Drury has explained his results by a different formula, namely,

$$\frac{\text{Output of urea}}{\text{Blood concentration}} = \text{a constant} \qquad 14$$

The injections were made in 0.9 per cent NaCl. This concentration of chloride is not altogether suitable as an interference can occur due to an

TABLE 8

C_B GRAMS PER 100 CC.	URINE VOLUME	$\sqrt{V}\ (C_u - C_B)$	OUTPUT / BLOOD CONCENTRATION	RATE OF INJECTION	PER CENT UREA IN INJECTED FLUID
	cc. per hour			*cc. per hour*	
0.377	68	1.01	0.96	100	2.5
0.482	116	0.96	1.09	100	2.5
0.755	265	0.85	1.31	180	5.0
1.004	162	1.07	0.88	180	5.0
1.305	156	1.10	0.76	180	5.0

In the third and fourth columns the figures are given as ratios of the average values.

TABLE 9

V	BODY WEIGHT	BODY TEMPERATURE	C_u GRAMS PER 100 CC.	C_B GRAMS PER 100 CC.	$\sqrt{V}\ (C_u - C_B$	OUTPUT GRAMS PER HOUR / C_B	NUMBER OF RESULTS
cc. per minute per kidney for 2 kgm. body weight	*kgm.*	°C.					
2.05	1.6	37	1.20	0.52	0.159	4.36	4
1.05	1.8	36	1.36	0.54	0.150	3.15	4
0.47	1.7	37	1.67	0.31	0.150	2.49	4
0.18	1.9	37	2.50	0.37	0.147	1.38	4

For the values of $\sqrt{V}(C_u - C_B)$, molecular concentrations have been used and corrections made for a temperature of 37°C. (Conway, 1925).

increase in plasma chloride (Conway and Kane, 1926). The greater the injection the less is this likely to occur as the urea increases much more rapidly in the plasma. In these experiments of Drury the interference has been apparently small. For the application of the equation

$$\sqrt{V}\ (C_u - C_B) = K_3 \qquad 3$$

we shall take as usual the results where the plasma figure exceeds 0.0375 M. The volume range for the 18 results from Drury's 5 rabbits is from 43 cc. to

265 cc. per hour and from 0.234 to 1.305 per cent for the plasma. The results from rabbit 1 in his tables show by far the widest range of blood concentration. In table 8 is given a comparison for this rabbit of the diffusion—secretion constant K_3 or $\sqrt{V}\,(C_u - C_B)$, and the expression used by Drury (1923) $\frac{\text{Output}}{\text{Blood concentration}}$, the figures being expressed as a ratio of the mean value over the range in both cases. It will be seen that the advantage is in favour of the constant K_3. The volume range being more evenly distributed in the 5 rabbits the effect of volume on the two constants may be expressed in the form of a statistical equation similar to those previously used. Taking the mean value of each constant as 100, the two equations are

$$K_{14} = 0.127\ V + 87 \qquad 15$$

$$K_3 = 0.040\ V + 95 \qquad 16$$

i.e., an increase of volume over the whole range 43 to 265 cc. per hour increases the constant in the diffusion equation 9 per cent and Drury's expression 30 per cent. Attention may be directed to the fact that a continuous injection of urea varying in quantity and composition was taking place in Drury's experiments. It is due to this factor that Drury's formula has any constancy at the high blood values, for the expression used by him altogether breaks down with the results of Conway and Kane (1926), Conway (1925), and Mayrs (1922). This may be seen in the 3rd column of table 3 with Mayrs' sulphate results, and in table 9 for the urea results of Conway and Kane (1926). For the individual rabbit where a large variation of volume is obtained with little change in the blood concentration, as much as eight times difference can occur in the ratio used by Drury (1923), the expression $\sqrt{V}\,(C_u - C_B)$ remaining constant.

The value of $\sqrt{V}\,(C_u - C_B)$ can be used only where the diffusion coefficient and the diffusion head remain constant, i.e., for a single substance when this altogether preponderates in the secretion. It is merely an abbreviation for $\sqrt{\frac{V}{D}}\,(C_u - C_B)$ or the still more general expression $\sqrt{\frac{V}{D}}\,\frac{C_u - C_B}{C_{max} - C_B}$. This last value as in equation 8 must be used in comparing the secretion constants of substances that differ both in their diffusion coefficients and "diffusion heads." The diffusion head $(C_{max} - C_B)$ for sulphate, urea, phosphate, etc., in the rabbit is probably the same when these singly are much increased in the blood. This is borne out by the fact as already shown that where sulphate and some other substance is injected, $\Sigma\sqrt{\frac{V}{D}}\,(C_u - C_B)$ is a constant. The diffusion head for sodium

chloride is however widely different, and there is some difference for urea when injected in 0.6 or 0.9 per cent NaCl, to when it is administered alone. This may be seen from the figures for the diffusion heads given in Section I. On the other hand the expression $\sqrt{\frac{V}{D}}\frac{C_u - C_B}{C_{max} - C_B}$ or $\Sigma\sqrt{\frac{V}{D}}\frac{C_u - C_B}{C_{max} - C_B}$ ($= K_s$) is independent of the diffusion head and should be a general constant, uninfluenced by the injected substance, the urine volume and the plasma concentration. That this is so will now be shown. The values for the diffusion heads used are those in Section I. The diffusion head for chloride is expressed in terms of total molecular concentration

TABLE 10

INJECTION	DIFFUSION HEAD	$\sqrt{\frac{V}{D}}(C_u - C_B)$ AT 39°C.	NUMBER OF RABBITS	NUMBER OF OBSERVATIONS	K_s	OBSERVER
Sodium sulphate.....	0.900	0.223	9	9	0.248 ±0.022	Mayrs (1922)
Glucose.....	0.900	0.209	5	8	0.232 ±0.029	Conway (1923)
Urea (in 0.9 per cent NaCl).....	0.773	0.178	5	18	0.230 ±0.028	Drury (1923)
Urea (in 0.6 per cent NaCl).....	0.773	0.186	14	16	0.241 ±0.026	Conway and Kane (1926)
Sodium chloride.....	0.280	0.062	8	27	0.221	Conway (1925)

In the table the nine observations of Mayrs include four experiments where creatinine was also injected—the creatinine concentrations of blood and urine being comparatively insignificant. The values for sodium chloride have been calculated using 1.25 C_B instead of C_B. Urine volume is expressed as in tables 6, 7, etc. Concentrations are expressed as molecular concentrations, allowance being made for ionization from the curves of electrical conductivity. The probable error has been used in column 6, its limitations in relation to normal distribution and number of results being recognized.

allowing for ionization from curves of the electrical conductivity. (In previous communications it may be here noted that chloride concentrations except when stated as percentages were expressed as mols NaCl per litre, or this unit × 2.) 0.90 M is used for Mayrs' sulphate results as the figure for the diffusion head is probably the same as for glucose and for urea, orally administered. The diffusion coefficients used are

Urea..... 0.97 (Heimbrodt, 1904)
Sodium chloride..... 1.02 (Heimbrodt, 1904)
Mannite..... 0.52 (Dombrowski, 1912)
Sulphate ..... 0.66 (Mellor, 1922)
Mannite for this purpose being taken as equivalent to glucose

These coefficients will be increased at body temperature but it is presumed that the increase will be similar for all. The results for the average values of K_8 are shown in table 10. Volume corrections for body weight have been introduced, a standard body weight of two kilos being chosen; also temperature corrections for a body temperature of 39°C. (Conway, 1925) have been included in the figures of Conway (1925) and Conway and Kane (1926), only those figures being used where both corrections could be made. The chloride value of the general constant, K_8, has been obtained from the expression $\sqrt{\frac{V}{D}}\frac{C_u - 1.25\,C_B}{C_{\max} - 1.25\,C_B}$, the value of $\sqrt{V}(C_u - 1.25\,C_B)$ being obtained from the curve through the values of C_u plotted against V (Conway, 1925) as owing to the very small differences of C_u and $1.25\,C_B$ at high values, and also to the fact that the factor 1.25 represents only an average relation

TABLE 11

MEANS	STANDARD DEVIATIONS	CORRELATIONS	THEORETICAL CORRELATION WITH PROBABLE ERROR FOR A NORMAL DISTRIBUTION	REGRESSION COEFFICIENTS
$x_1 = 0.240$	$\sigma_1 = 0.052$	$r_{12} = +0.07$	$r_{12} = \text{zero} \pm 0.08$	$b_{12} = +0.0045$
$x_2 = 1.023$	$\sigma_2 = 0.836$	$r_{13} = +0.06$	$r_{13} = \text{zero} \pm 0.08$	$b_{13} = +0.077$
$x_3 = 0.086$	$\sigma_3 = 0.042$	$r_{23} = +0.21$		

$x_1 = \sqrt{\frac{V}{D}}\frac{C_u - C_B}{C_{\max} - C_B}$ or $\Sigma\sqrt{\frac{V}{D}}\frac{C_u - C_B}{C_{\max} - C_B}$

$x_2 = V$, urine volume in cubic centimeters per minute per kidney.

$x_3 = C_B$ (Plasma concentration of injected substance or substances expressed as in previous table).

Number of observation sets = 75.

after large chloride injections, this procedure is better than the summation of the individual results. Although the "diffusion heads" of table 10 are to some extent derived values, the table gives sufficient indication that $\sqrt{\frac{V}{D}}\frac{C_u - C_B}{C_{\max} - C_B}$ is a general constant independent of the injected substance. It will now be shown using standard statistical methods (Yule, 1924) that this expression or $\Sigma\sqrt{\frac{V}{D}}\frac{C_u - C_B}{C_{\max} - C_B}$ is independent of urine volume and plasma concentration. For the calculation 75 sets of figures are available, 51 being of independent observers. The chloride observations have been omitted for the reasons given above. The figures used comprise 33 sets of Mayrs (1922, 1923), 18 of Drury (1923), 16 of Conway and Kane (1926) and 8 of Conway (1925). The chief statistical data are given in table 11. The correlation coefficients of K_8 with V and C_B are equivalent

to complete independence with the number of figures used. Expressing the results as linear statistical equations we have

$$K_s = 0.0045\ V + 0.235 \qquad 17$$

$$K_s = 0.077\ C_B + 0.233 \qquad 18$$

The equations show that a forty times increase of volume, from 0.1–4.0 cc. per minute changes the value of K_s only 7.5 per cent and that an increase to about 6 times the plasma concentration from 0.0375 to 0.221 (molecular concentrations), causes an increase in K_s of only 6.1 per cent. These effects are within the error limits expected and the results prove the statement that the expression $\sqrt{\frac{V}{D}}\frac{C_u - C_B}{C_{\max} - C_B}$ is independent of the volume and the plasma concentration beyond the lower limits assigned to these values.

IV. The diffusion equation at low plasma concentrations for non-threshold substances. Substances are regarded here as non-threshold, if they never appear in the urine in less concentration than in the blood, the threshold itself being taken as the highest value or limit of C_B where $C_u < C_B$. (If however the threshold of a substance is small compared with its ordinary blood values, it may be here treated as non-threshold.)

Urea is non-threshold in the above sense, as it is never found in the urine in less concentration than in the plasma (see also fig. 2), and unlike ammonia (Nash and Benedict, 1921), or creatinine (Behre and Benedict, 1922) it is not manufactured in the kidney to an extent appreciable when compared with its secretion.

A large number of estimations on human subjects have been made by various observers of the urinary and blood urea. At the normal ranges of blood concentration at constant body weight it is secreted according to equation 6, as first pointed out by Austin, Stillman and Van Slyke (1921), subsequent to the work of Ambard (1910a, 1910b) who had proposed a different relation.

In confirming equation 6, we have applied mathematical methods of a stricter kind to show the effect of C_B (blood concentration) and V (urine volume, or $\frac{1}{t}$) on C_u (urine concentration) or on VC_u (output).

The formulae proposed for the excretion of urea can be stated in the general form

$$C_u = V^a \times C_B{}^b \times W^c \times \text{a constant} \qquad 19$$

W being the body weight. The equation may be written

$$\text{Log } C_u = a \text{ Log } V + b \text{ Log } C_B + c \text{ Log } W + K_{20} \qquad 20$$

Here C_u is to be regarded as a variable dependent on V, C_B and W. By adding log V to both sides we could write the equation with output as the dependent variable.

$$\text{Log (output)} = d \text{ Log } V + b \text{ Log } C_B + c \text{ Log } W + K_{21} \qquad 21$$

but it is more rational to consider concentrations rather than output, since the interaction of cell and environment at any moment must be a question of concentrations whether we are considering diffusion, energy change, or limitation of the concentrative mechanism. It may be pointed out that exactly the same amount of urea could be excreted with practically no energy expenditure or with a comparatively large amount depending on the ratio $\frac{C_u}{C_B}$. The coefficients a, b and c in the formula of Austin, Stillman, and Van Slyke are -0.50, 1.00 and 0.50. Ambard's combined formula gives the coefficients -0.66, 1.33 and 0.67. McLean's formula (1915) being another variation of Ambard's gives the same coefficients. Walker and Rowe (1927b) cited an equation which does not consider the effect of volume on output and may be written

$$\text{Log } C_B = K + 0.72 \text{ Log } VC_u$$

The coefficients here are $a = -1.00$ and $b = 1.39$, the blood concentration having the same effect on output as in Ambard's formula. (These two workers after showing the great effect of C_B on output, in showing the effect of volume, plot the average output against volume, without taking C_B into consideration. In consequence of this, conclusions drawn from the curve cannot be accurate.)

In calculating the best values of the coefficients from the principle of least squares standard statistical methods of multiple correlation have been used (Yule, 1924) depending on an extension of the minimum square error principle. One hundred and seventy-seven groups of corresponding values of C_u, C_B, V and W, 708 observations in all, have been taken from the literature. No principle of selection for these figures was adopted except that they were taken within a volume limit of four litres per day, approximately this limit being assigned for the application of equation 6. The figures include the following number of sets of observations by different observers.

Addis and Watanabe (1916)	= 92
Walker and Rowe (1927a)	= 47
Austin, Stillman and Van Slyke (1921)	= 23
McLean (as quoted by Austin etc., 1921)	= 15

These figures include groups on fasting subjects, i.e., 27 sets of Walker and Rowe, others where the volume of the urine was increased by water intake (Austin, etc.), and others where there was apparently much variety in the water and nitrogen intake.

In three of the observations of Addis and Watanabe the value of W not being given—the average value of W is substituted. From the data obtained (see table 12) the general equation showing the influence on log C_u of the other variables may be written.

$$\text{Log } C_u = -0.51 \log V + 0.95 \log C_B + 0.32 \log W + 0.697 \qquad 22$$

The correlation coefficient between log C_u and whole expression on right is 0.85, which is satisfactory. (Elaborate tests for the linearity of this

TABLE 12

MEANS	STANDARD DEVIATIONS	CORRELATIONS OF ZERO ORDER	CORRELATIONS OF SECOND ORDER	REGRESSION COEFFICIENTS USED IN TEXT
$x_1 = 1.278$	$\sigma_1 = 0.206$	$r_{12} = -0.417$	$r_{12.34} = -0.73 \pm 0.02$	$b_{12.34} = -0.51 \pm 0.02$
$x_2 = 1.717$	$\sigma_2 = 0.234$	$r_{13} = +0.625$	$r_{13.24} = +0.81 \pm 0.02$	$b_{13.24} = +0.95 \pm 0.03$
$x_3 = 1.536$	$\sigma_3 = 0.164$	$r_{23} = +0.214$	$r_{23.14} = +0.68 \pm 0.03$	$b_{14.23} = +0.32 \pm 0.11$
$x_4 = 1.993$	$\sigma_4 = 0.049$	$r_{34} = -0.082$	$r_{14.23} = +0.14 \pm 0.05$	
		$r_{14} = -0.013$	$r_{24.13} = +0.14 \pm 0.05$	
		$r_{24} = +0.045$	$r_{34.12} = -0.17 \pm 0.05$	

$x_1 = \text{Log } C_u$ (C_u = grams urea per litre of urine)
$x_2 = \text{Log } V$ (V = cc. per hour of total urine)
$x_3 = \text{Log } C_B$ (C_B = milligrams urea per 100 cc. blood)
$x_4 = \text{Log } W$ ($W = \frac{\text{Body weight in kilos}}{70}$)

Number of observation sets = 177.
About 15 class divisions were used to cover the range of each variable.

partial regression equation have been thought unnecessary and beyond the scope of the present work.) The result establishes the coefficients a, b and c as -0.51 ± 0.02, $+0.95 \pm 0.03$, and $+0.32 \pm 0.11$ so that the formula expressing the influence of V, C_B and W on C_u can be written

$$\sqrt{\frac{V}{W^{\frac{1}{3}}}} \frac{C_u}{C_B} = K_{23} \qquad 23$$

In this formula it will be noted that the function of the weight follows a surface relation though as may be seen from the very low correlations of W, and the wide error limits of the coefficient c this is of little importance. In fact very little error will result from the total omission of W. On limiting the volume to 3 litres per day the coefficients a, b and c are not

appreciably different so that four litres per day seems to be within the upper limit of volume, as will also be seen from table 13 and figure 1. Taking the influence of volume alone at constant blood concentration and body weight the statistical formula may be written

$$\text{Log } C_u = K_{24} - 0.51 \log V \qquad 24$$

The addition of log V to each side will make no difference to the result, i.e.,

$$\text{Log } VC_u = K_{24} + 0.49 \log V \qquad 25$$

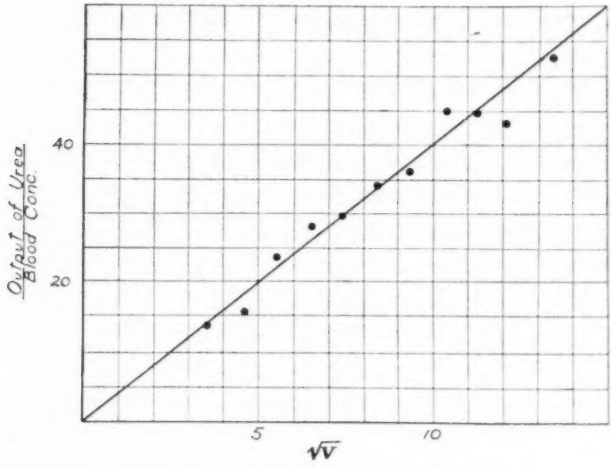

Fig. 1. Units as in tables 12 and 13. Observations as in table 13

The output at constant C_B and W is thus directly proportional to the square root of the volume. The influence of C_B may be represented at constant volume and body weight as

$$\text{Log } VC_u = K_{25} + 0.95 \log C_B \qquad 26$$

i.e., the output at constant volume and body weight is directly proportional to the blood concentration.

The result of the statistical calculations is decisive in establishing the diffusion formula $\sqrt{\frac{1}{t}}\frac{C_u}{C_B} = K_6$ as expressing the secretion of urea in the normal human subject (up to the volume limit), where the urine volume and the blood concentration are regarded as independent variables

and the urine concentration or output as the dependent variable. (See also table 13, figs. 1 and 2.) Another illustration of this result is given by the correlations of $\log \frac{V^{0.51} C_u}{C^{0.95} W^{0.32}}$ with the two variables $\log V$ and $\log C_B$. These correlations are -0.025 and -0.052, and the theoretical requirement being zero $\pm 0{,}05$, are equivalent to complete independence. Similar correlations obtained for Ambard's expression, are -0.14 and $+0.44$.

Beyond the volume limit of approximately 4 litres the output $\left(\frac{VC_u}{100}\right)$ becomes independent of the volume (Austin, etc., 1921; Addis and Drury, 1923c), a relation which we shall consider in section V. The appearance

TABLE 13

RANGE OF $\sqrt{V}$	$\sqrt{V}$	$\frac{V^{0.5} C_u}{C_B W^{0.32}}$	$\frac{\text{OUTPUT PER HOUR IN MGM.}}{C_B}$	C_B	NUMBER OF OBSERVATION SETS
3.0– 4.0	3.5	3.93	14.0	34.3	2
4.0– 5.0	4.6	3.56	15.9	37.6	14
5.0– 6.0	5.5	4.42	23.9	27.6	24
6.0– 7.0	6.5	4.35	28.2	33.2	41
7.0– 8.0	7.4	4.09	29.7	37.0	34
8.0– 9.0	8.4	4.06	34.1	44.0	25
9.0–10.0	9.3	3.87	36.1	48.0	12
10.0–11.0	10.4	4.28	44.9	52.8	12
11.0–12.0	11.3	3.83	44.7	36.5	6
12.0–13.0	12.1	3.56	43.2	29.7	7
13.0–14.0	13.5	3.88	52.6	30.4	7

The units used are the same as for table 12, the observation sets being also from the same source.

of this relation has led some observers to believe that the output at constant C_B is independent of the whole range of urine volume. Here, according to this view, we must suppose that the high correlation of output and the volume up to a certain limit depends on some third factor or factors with which both are highly correlated, though even in such a case there would appear no reason for the square root relation. These factors as advanced by Addis and Drury (1923c) are water, saline and nitrogen intake. No effect has been shown by the intake of water on the secretion of urea other than that due to the volume increase so that we are left to consider the evidence in connection with saline and nitrogen intake. The evidence given for saline intake (1 per cent) depends on very few observations which are also liable to another explanation and the question at issue has been answered decisively by the recent very numerous observations

described by H. L. White (1927). He draws the conclusion that the output of urea is independent of the sodium chloride content of the ingested water. As to the effect of nitrogen intake, the results of Addis and Drury (1923b) may be summarized in the statement that an average increase in the ratio $\frac{\text{urea output}}{\text{Blood urea}}$, in the neighbourhood of 25 per cent, is produced by nitrogen intake (urea, or nitrogenous food), at high urine volumes where the ratio is independent of the volume—and thus cannot be explained by

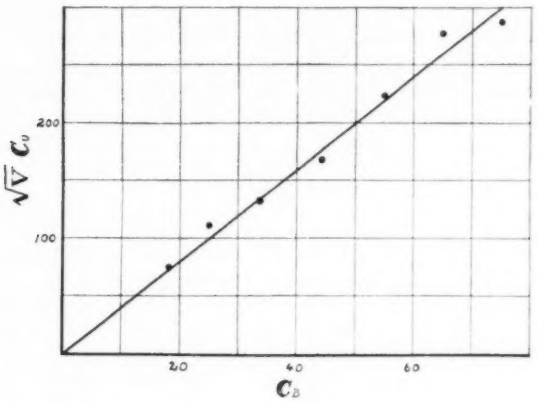

Fig. 2. Units as in tables 12 and 13. Observations as in table 13. It will be seen from the figure that urea has no threshold. Compare Adolph (1925), also Addis and Drury (1923a). It will be noticed that the linear curves of output $\frac{V\,C_u}{100}$ given by the latter observers for observations at high urine volumes where equation 7 applies, also pass through the origin. Adolph who applies equation 7 at low volumes where it does not hold, obtains a false threshold.

a volume increase. This result is somewhat paradoxical because the ratio $\frac{\text{output}}{\text{Blood urea}}$ is represented by the same workers as constant over the widest normal range of blood area (1923a)—yet what can increase the blood urea here except intake of urea or nitrogenous food? A small increase of the ratio $\frac{\text{urea output}}{\text{Blood urea}}$ at high urine volumes can be explained as will be seen in section V by a possible increase in kidney efficiency, but even if we accept the increase from the standpoint of Addis and Drury, as entering into the data here examined, it can enter only partially, since the volume increase was only partly due to nitrogenous intake (see tables

12 and 13), but in any case a possible effect in the neighbourhood of 25 per cent cannot be used to explain an actual result of upwards of ten times that amount, as may be seen from table 13 (and fig. 1).

It will also be noticed from table 13 that there is a decided decrease in the average blood concentration at the range of higher urine volumes so that it would seem that the effect of nitrogen intake cannot be used at all to explain the results. The evidence brought by these workers from rabbit experiments (1923c) rests on a misapprehension as to the range of applicability of their formula $\frac{\text{urine output}}{\text{Blood urea}}$ = a constant, as will be seen from section III of present paper. Average errors of many hundreds per cent can be introduced by accepting this formula when C_B is high.

Reference may be made here to another paper on the same subject by Bourquin and Laughton (1925). These workers experimented on dogs, examining the effect of diuresis on the urea output. In many of the

TABLE 14

PERIOD	V (URINE VOLUME)	$\sqrt{V}\,C_u$			$V \times C_u$ FOR CREATININE
		Urea nitrogen	Phosphorus	Sulphur	
I	103	50.4	2.37	3.42	53.8
II	92	42.0	1.78	2.31	52.3
III	117	39.2	2.32	1.94	52.8
IV	83	37.9	2.57	1.99	50.7

Units used are the same as in tables 12 and 13, C_u being expressed as grams per litre.

experiments injections of 40 per cent sodium sulphate were given. The results obtained can be readily explained in the light of the evidence examined in section III of present paper.

The excretion of phosphate and sulphate at the low, normal blood concentration, should from the theory proposed follow the same course of excretion as urea. It seems (Wigglesworth and Woodrow, 1923) that at high urine volumes the phosphate excretion is similar to urea being expressed by the relation $\frac{\text{Output}}{\text{Blood conc.}}$ = a constant. Below a urine volume of 4 litres, it appears from the experiments of White (1926) that phosphate and sulphate are likewise following the diffusion equation 6. These latter experiments are of interest in showing that where a diuresis is produced by the mere fact of recumbency the excretion follows the formula $\sqrt{V}\,C_u = k$, at constant C_B. In these observations of White the subject took no food or water after 10 p.m. on the evening preceding the day of the experiment. At 8 a.m. he drank 200 cc. of tap water, voided and discarded

the urine. He then stood or lay for two hours, voiding urine at the end of each two-hour period having at the beginning of each drunk 200 cc. of water. The order of lying and standing periods was varied in the various experiments, and in each experimental day there were four consecutive two-hour periods, of which table 14 gives a summary (A few of the urine volumes exceed 4 litres per day, these have been reduced to this volume on the basis that beyond it the output becomes constant.)

It will be seen from the table that the values of $V^{\frac{1}{2}}C_u$ for urea and sulphate fall as the time progresses, this no doubt being due to a corresponding fall in the plasma concentrations. If we confine ourselves to the second and third or to the third and fourth periods and obtain the average values for the recumbent and standing times, we shall get a contrast of values of $\sqrt{V}\ C_u$ at the same average blood concentration, but different average volumes. The results are summarized in table 15 as ratios of the average

TABLE 15

PERIODS FROM WHICH THE FIGURES ARE TAKEN	RATIO → V (URINE VOLUME) $\frac{\text{RECUMBENT VALUES}}{\text{STANDING VALUES}}$	$\sqrt{V}\ C_u$—RATIO OF $\frac{\text{RECUMBENT VALUES}}{\text{STANDING VALUES}}$		
		Ratio for urea nitrogen	Ratio for phosphorus	Ratio for sulphur
Third and fourth.....	2.42	1.00	0.91	0.97
Second and third.....	2.81	1.01	1.05	0.86

values for the recumbent and standing position. It will be seen that there is an increase in the average volume of 142 per cent and 181 per cent in recumbency, but the values of $\sqrt{V}\ C_u$ remain constant. This is particularly true of urea and while the figures for P and S are not quite so good, it is obvious that they are following a similar relation.

V. THE FORMULA $\frac{\text{OUTPUT}}{\text{BLOOD CONCENTRATION}}$ = A CONSTANT, FROM THE DIFFUSION STANDPOINT. This formula may be written

$$\frac{VC_u}{C_B} = K_7 \qquad 7$$

and applies at normal blood concentrations when the urine volume exceeds about four litres per day. If we introduce the conception of a limit of secretion energy, for a given blood concentration, it can be deduced from the diffusion secretion theory that the output of urea will become constant beyond a certain urine rate.

The formula expressing the energy required to excrete urea or other substance may be written

$$W = VC_u \left(\text{Log} \frac{C_u}{C_B} - \frac{C_u - C_B}{C_u} \right) \times K_{27} \qquad 27$$

where W is the work done in secreting $\frac{VC_u}{100}$ grams of urea, C_u being the percentage urine concentration. From the diffusion-secretion theory the ratio $\frac{C_u}{C_B}$ in the right hand expression does not express the true concentration difference owing to diffusion gradients and the fact that C_u merely gives the final concentration in the tubule lumen. The ratio to be used in the formula should express the ratio of the concentrations in the stationary film of urine and the adjacent region of the tubule cells. This ratio will be a constant over the lower ranges of C_B and up to four litres of urine volume, and will represent the maximum concentrating power of the kidney. The ratio would seem from graphs of concentration at the lowest rates of flow to be roughly 100, though its exact value does not affect the argument. Writing

$$W = VC_u \left\{ \text{Log } P - \left(1 - \frac{1}{P} \right) \right\} \times K_{28} \qquad 28$$

where the ratio P is 100 we can neglect $\frac{1}{P}$ as compared with unity so that the expression becomes

$$W = VC_u \text{ Log} \frac{P}{e} \times K_{28} \qquad 29$$

when the maximum work is reached with increasing urine rate, the expression $VC_u \log \frac{P}{e}$ becomes constant. Though P must now fall with increasing value of V, it can be readily calculated that a further increase of 300 per cent in V causes an increase in the output of only 17 per cent, i.e., the output becomes largely constant. The calculation makes use of the diffusion relation

$$\frac{\sqrt{V}\, C_u}{P} = \frac{\sqrt{V_1}\, C_{u1}}{P_1}$$

To deduce further that for similar volume changes the output divided by C_B remains constant, when C_B varies, it would be necessary to assume that the available energy of the kidney, other things being equal, was propor-

tional to the urea concentration of the blood over the normal range. The useful work done by the kidney increases directly with this concentration. The assumption that the available energy varies with C_B (within limits) is thus a reasonable one, for if it does not so vary, it would appear to be largely wasted at the lower and more usual values of C_B. The question however has not been experimentally determined in detail.

In experiments on rabbits which had received large injections of urea, glucose, sulphate, etc., an upper volume limit for the application of the diffusion formula was not found. This may be interpreted in the sense of the above conception of a limit to the available energy. Beyond a certain value of C_B, $C_{\max}$ (or more strictly, $C_{\max} - C_B$) ceases to rise and become constant. From this point the value of the ratio $\frac{C_{\max}}{C_B}$ the logarithm of which occurs in the energy equation (as treated from the diffusion standpoint) falls rapidly with increasing C_B and the volume of urine at which the kidney reaches the limit of its powers is correspondingly increased.

Mention may be made here of the possibility of the mean concentration in the tubule capillaries being seriously diminished by the secretion.

This possibility would arise where the blood urea concentration was in the normal range and the urine rate high, that is, for the condition discussed in the present section. If we assume as probable Rehberg's calculation (1926) of the blood flow through the human kidney from comparative figures, the mean fall would however be only about four per cent.

The formula under discussion (equation 7) has been explained from the usual filtration absorption view (e.g., Marshall, 1926) as due to a constant output of glomerular fluid. But in this case it would only hold if no urea were reabsorbed, and it must now be assumed by supporters of the water absorption theory (e.g., Rehberg, 1926) that urea is extensively reabsorbed in the human subject and at high rates of urine flow.

A general discussion of the diffusion secretion theory in its bearing on other lines of renal investigation will be given in the subsequent communication. In conclusion, some general reasons may be given why other formulae arise for the excretion of urea in the human subject differing from equations 6 and 7.

1. The attempt to bring all the data under one formula not recognizing an upper limit of volume as shown by Austin, Stillman and Van Slyke (1921) will necessarily lead to a multiplicity of formulae depending on the relative number of data taken beyond the volume limit to the number taken below it. The validity of equation 6 up to the volume limit is sufficiently proved here by table 13, by figures 1 and 2 and by the statistical calculations. Equation 7 is proved by the observations of Austin, etc. (1921) and the numerous results of Addis and Drury (1923a, 1923c).

2. Any formula proposed for the data in the literature must be based on statistical calculations, and the statistical equations which take V, C_u, C_B and W as the variables in question in the manner of equation 19 will not be same as these are in turn regarded as the dependent variable. The selection of the dependent variable is naturally governed by logical considerations; for example, it would be absurd to suppose that body weight is dependent on the blood concentration. The only rational dependent variable is here either the urine concentration or the output, being a resultant of the blood concentration, the urine rate, and body weight. These latter are taken in this sense as independent variables (see equations 20, 21). It may here be mentioned that Ambard has regarded urine concentration as an independent variable in its influence on output.

3. Just as any formula advanced must imply an underlying statistical equation, such an equation may be very inadequate. The equations

$$\text{Log (output) or Log } VC_u = \text{a Log } C_B + \text{a constant}$$

$$\text{Log (output) or Log } VC_u = \text{a Log } V + \text{a constant}$$

are an example, unless it is understood that the first is taken at constant urine volume (or at volumes in excess of about 170 cc. per hour) and the second at constant blood concentration. Examples of these two forms of inadequacy may be found in the literature.

It might be urged that the statistical equation here arrived at for secretion in the human subject

$$\text{Log } VC_u = 0.5 \log V + 1.0 \log C_B + 0.3 \text{ Log } W + K$$

is also to some extent inadequate since log of output (or log VC_u) is not in particular instances completely determined by the variables on the right hand side. This inadequacy is of no account for the present discussion, since first, the unknown extra variables necessary to completely determine the output have but a small effect compared with those discussed, and secondly, they act independently of V and C_B.

This independence is shown by a number of facts, of which the following may be mentioned:

a. Individual subjects may be found where over a wide range of urine rate (up to the volume limit) the output is closely determined by V and C_B (see Austin's figures, Austin, etc., 1921) yet the coefficients of the variables in the above equation remain unchanged.

b. In conditions where the output is determined very closely by C_B (the urine volume exceeding 4 litres per day; Addis and Drury, 1923a) the coefficient of C_B remains unchanged though the range of C_B is wide.

c. If we compare excretion by the rabbit at high blood concentration, very large variations of urine rate may be obtained in the same rabbit.

These variations often exceed a thirty times increase (Conway, 1925; Conway and Kane, 1926), the value of $\sqrt{V}(C_u - C_B)$ remaining practically constant. Here the volume still enters as its square root function.

d. Lastly may be mentioned the fact that the frequency distribution of the secretion constant is independent of V and C_B. As will be seen in the following communication this frequency distribution throws some light on the nature of the extra variables.

SUMMARY

1. Evidence is given that the diffusion formulae described in a previous communication (Conway and Kane, 1926) hold for diffusion into a stream of water passing through a capillary tube of a kind which may be held to resemble conditions in the kidney tubule.

2. The diffusion-secretion formula $\sqrt{\frac{1}{t}}(C_u - C_B) =$ a constant, applying to glucose, urea and with a modification to sodium chloride, at high blood values (Conway, 1925; Conway and Kane, 1926) has been shown from the figures of Mayrs to apply to sodium sulphate. It has been also shown that the expression $\sqrt{\frac{1}{t \times D}} \frac{C_u - C_B}{C_{\max} - C_B}$ (or where more than one substance is injected $\Sigma\sqrt{\frac{1}{t \times D}} \frac{C_u - C_B}{C_{\max} - C_B}$) of which $\sqrt{\frac{1}{t}}(C_u - C_B)$ is an abbreviation, is a general constant for all substances when the plasma concentration exceeds a certain value. Here C_u and C_B are the concentrations of urine and plasma, t is the time of unit volume of urine, D is the diffusion coefficient and $C_{\max}$ the highest value of C_u as V or $\frac{1}{t}$ is indefinitely diminished. In the above expressions $C_u - C_B$ is to be regarded as the output of the tubules into unit volume of urine and $C_{\max} - C_B$ as the "diffusion head." Other formulae hitherto proposed for renal excretion are very inapplicable at high blood values. Where in very special conditions Drury (1923) has found an agreement with another equation, it is shown that the diffusion secretion formula applies even better.

3. Evidence for the diffusion-secretion formula

$$\sqrt{\frac{1}{t}} \frac{C_u}{C_B} = \text{a constant}$$

applying to urea excretion in the human subject has been subjected to a statistical analysis, and it is shown to be the true relation up to a urine volume of approximately 4 litres per day.

Evidence is also presented from the work of White, etc., that sulphate and phosphate follow the same relation in the human subject.

4. Reasons have been advanced for the constancy of $\frac{\text{output of urea}}{\text{Blood conc.}}$ at high urine volumes and normal blood concentrations, being possibly due to a limit of available renal energy, reached at a urine volume in the region of 4 litres per day.

5. The formulae for renal secretion as here demonstrated derive according to the theory of Conway and Kane (1926) from the fundamental diffusion relation

Output from tubules into unit volume of glomerular fluid $= \sqrt{t} \times$ "the diffusion head" $\times$ a constant.

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THE EQUATION EXPRESSING THE EXCRETION OF A DIURETIC AND ITS RELATION TO DIFFUSION PROCESSES

Part III

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I. According to the theory presented in previous communications where the chloride of the urine is less than in the plasma it is necessary to suppose either that the chloride in the glomerular fluid is diminished, or that the chloride is reabsorbed in the tubules. Wearn and Richards (1925) have presented direct evidence for the reabsorption of chloride in the frog. This has been confirmed by White and Schmitt (1926) for the Necturus. The absorption of chloride according to the diffusion secretion theory would take place by a reversal of the secretion process. Just as in the theory presented in the previous communications it was taken that water is not secreted from the tubule cells with the urea, etc., so here the chloride is to be considered as absorbed without any absorption of water. There is rather a diffusion of the solutes towards a state of balance on each side of a cell or membrane, a balance such as would occur for example when iodine diffuses from water into chloroform.

Upon any general theory of urinary secretion an equilibrium may be supposed to be reached as the urine approaches zero rate, the secretion conditions being otherwise unchanged. The transformation of the glomerular fluid considered as a physico-chemical process in actual cases must be regarded in its relation to such a potential equilibrium. If this balance in a hypothetical case resulted in a urine of 0.05 per cent chloride the absorption theory most favoured of recent years (Cushny, 1926) would maintain that it is reached by the continuous abstraction from the original glomerular fluid of a solution of fixed chloride concentration along the absorbing region of the tubule. Let us consider the implications of this idea. At the beginning of such a process the chloride and the water are being absorbed at rates closely proportional to their concentrations. But as the chloride concentration becomes progressively reduced the concentration of chloride absorbed from the lumen remains unaltered and consequently in relation to the concentration of the fluid from which it is being

extracted enormously increased. In other words the passage of chloride into the cells relative to its concentration in lumen is greatest when the chloride in lumen is least, and is always so nicely adjusted to the water absorption that the absorbed fluid is of constant concentration. Stated in this form the improbability of the conception is clear, and the improbability applies to all views which suppose the absorbed fluid constant in concentration from the top to the bottom of the absorbing region.

Assuming for the moment an absorption of fluid it would seem a priori more probable that this absorbed fluid should bear some relation to the concentrations prevailing in that from which the absorption is taking place and progressively vary as the urine, passing along the tubule, reaches closer to the theoretical equilibrium. We could suppose that instead of fluid of constant composition being absorbed, chloride is being absorbed at a definite rate per unit concentration down the tubule. As the final urinary chloride concentration is sometimes above and sometimes below the plasma chloride the ratio of the rates of absorption of chloride and water must vary accordingly. The chloride rate per unit of concentration must at times be greater than that of the water with the result that the urinary chloride progressively diminishes and when the reverse holds the chloride becomes concentrated in the urine. The relative rate of the chloride absorption must depend on the plasma since this determines largely whether the urine has a high or low concentration. We could regard the threshold as the plasma concentration when chloride and water absorption rates are equal. It may be granted then that the water absorption view implies a chloride absorption relative to the water of all degrees of difference over a wide range, and is regulated by the concentration of the plasma.

The diffusion secretion theory states in contrast to this that what the plasma chloride influences is the rate and direction of chloride diffusion alone, no water being absorbed. That is to say, in the absorption of chloride by the tubules, there is only one control—that for chloride, and the facts presented in this paper are in harmony with this view and not with the other. The site for control of water output is placed at the glomerulus.

It may be necessary to suppose with this theory that the chloride is absorbed by a different region of tubule than that at which secretion is occurring. This however might well find an anatomical basis in the kidney structure.

II. The chloride threshold. The chloride threshold is here defined as the upper limit of C_B, at which C_u (urine concentration) is $< C_B$ (plasma concentration) for all rates of urine flow. At this limit, and in terms of chloride concentrations the threshold, the plasma, the urine and the glomerular fluid may be regarded as having equal values. We have considered in dealing with the chloride secretion (Conway and Kane, 1926; Conway,

1925) evidence for the glomerular concentration being increased over the plasma after large chloride injections. In the present investigations after injections of glucose, sulphate, etc.—we find it better to regard the glomerular fluid and plasma concentration as having the same value. The conditions in the blood are very different, the concentrations of glucose or sulphate, etc., being very high and the chloride concentrations comparatively low (it is chloride concentrations only up to the threshold value that are of significance for the present study). Previously (Conway, 1925) the reverse condition was present in the blood—the chloride being very high through injections and other substances low, as none were injected.

Whether without any injection the glomerular fluid in the mammal will show concentration of chloride by the glomerulus around the threshold value we have no evidence to decide, but deem it better, without this evidence to abide by the above definition as covering such cases.

We have chosen for the definition of the threshold a limit of C_B rather than the simple equality $T = C_B = C_u$, since the equality of C_B and C_u obtains in the dead kidney. Previous determinations of the chloride threshold in man such as those of Ambard and Weill (1912) and McLean (1915) have proceeded from the standpoint that the chloride threshold of the plasma is that at which chloride is practically absent from the urine. This however is an arbitrary position as the chloride content of the urine depends not only on the plasma concentration but also largely upon the urine rate. In the rabbit for example even at plasma values far below the true threshold—lower even than 0.4 per cent—on sufficiently increasing the urine rate, chloride, though never reaching the plasma concentration appears in the urine in very appreciable concentrations (the relation of urine volume to absorption will be seen in fig. 1 which is described in section IV). McLean (1915) has published the results of many urinary and plasma analysis in normal man and places the average normal chloride threshold at 0.562 per cent confirming the conclusions of Ambard and Weill. From his 29 sets of analyses for normal subjects and assuming that for a small range above and below the threshold the linear relation

$$C_u = a\,C_B + b \qquad 1$$

applies where C_u is a variable dependent on C_B we have determined from his figures by statistical methods the value of the true threshold. The equation giving the best value of a from the principle of least squares is

$$C_u = 15.61\,C_B - 8.437 \qquad 2$$

from which, when

$$C_u = C_B = T,\ T \text{ is } 0.577$$

(if calculated using the probable error of the regression coefficient, T may be given as 0.575 ± 0.007). The coefficient of C_B will largely depend on the average urine volume and strictly speaking, owing to the variability of V, this equation (and similar equations in the context) are of little value except as giving a figure for the threshold. We have collected similar figures from 19 anesthetized rabbits which apart from the anesthetic (urethane, administered in solution by stomach tube) had received no previous injections. Two of the rabbits showed very aberrant values for the thresh-

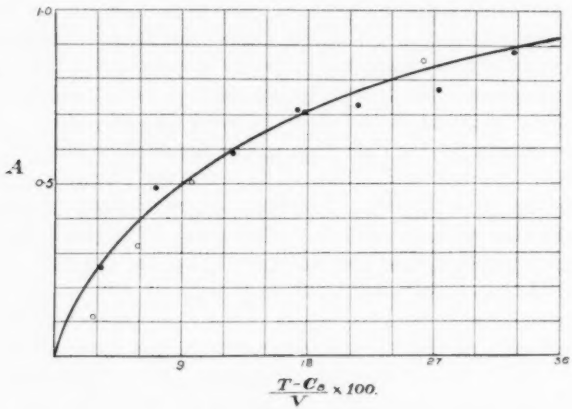

Fig. 1. Figures from tables 6 and 7. Curve from table 10

old, being > 0.630 (? nephritic). These have not been considered, and the equation obtained is then

$$C_u = 31.86\, C_B - 17.72 \qquad 3$$

from which the threshold $= 0.574$, i.e., practically identical with the value obtained from McLean's figures for man. Here the mean value of $C_B =$ 0.597 per cent, of C_u, 1.296 per cent and of urine volume 0.083 cc. per minute per kidney or 0.228 litre per day total urine. The plasma chloride analyses of these rabbit experiments and those to be described were carried out by the Austin and Van Slyke method (1920) the proteins being first precipitated by picric acid, and by Whitehorn's method (1921) for the urine. (Whitehorn's method was also used for a small proportion of the plasma determinations.) For the plasma analyses 5 cc. or more of blood were taken from a carotid cannula, 2 cc. of plasma being subsequently used. The blood was received into a tube containing a little powdered oxalate. The rest of the tube was filled with alveolar air and corked. The blood was then mixed and centrifuged. With each plasma analysis (by the

Austin and Van Slyke method) one or more controls of 0.5 per cent standard chloride solution went through the complete process. The result of the analyses of 36 of these controls throughout the experiments may be stated as 0.501 ± 0.005.

III. THE EFFECT OF INJECTIONS (GLUCOSE, SULPHATE AND UREA) ON THE CHLORIDE THRESHOLD. *Glucose:* Large injections of 5 per cent glucose were given through a jugular cannula to rabbits anesthetized with urethane the injection being given over a period of 5 to 10 minutes Urine and blood samples were then collected—the urine from a bladder cannula and the blood from a carotid. Two per cent saline was then injected in small quantities (5 to 20 cc.) at a time—after each injection samples of urine and blood being taken. Results were collected in this manner from

TABLE 1

NUMBER OF EXPERIMENT	INJECTION OF 5 PER CENT GLUCOSE	THRESHOLD	C_B BEFORE INJECTION	URINE VOLUME AROUND THRESHOLD
	cc.			*cc. per minute per kidney*
1	114	0.573	0.593	0.2
2	133	0.590	0.583	0.4
3	125	0.570	0.598	0.6
4	143	0.560	0.573	0.6
5	143	0.563		1.4
6	150	0.598		0.6
7	70	0.569		1.2
8	200	0.575	0.590	0.8
9	57	>0.640	0.656	

C_B = percentage concentration of NaCl in plasma. Threshold also expressed as percentage concentration.

9 rabbits. For each rabbit a curve was drawn through the urine concentrations plotted against the plasma concentrations. Where this curve cuts the line of equal blood and urine values was taken as the threshold. Table 1 illustrates the results obtained. For one rabbit the threshold was abnormally high, the plasma chloride being also very high before injection of glucose. This aberrant result may be due to nephritis. For the remaining 8 rabbits the average threshold was 0.575 per cent after an average injection of 135 cc. of 5 per cent glucose. Other methods were also used for determining the threshold after glucose injections.

The equation form was used as before, the values of C_B being restricted to the normal range of plasma chloride (0.570–0.640); the resulting equation is

$$C_u = 5.60\ C_B - 2.665 \qquad 4$$

from which the threshold is 0.579 (or 0.577 ± 0.007, considering the probable error). In another method the C_u and C_B values immediately above and below the threshold were averaged giving the following figures:

URINE VOLUME		C_u	C_B
cc. per minute per kidney for 2 kilos body weight			
Above	0.691	0.674	0.585
Below	0.746	0.379	0.556

Plotting these results of C_u and C_B we obtain for the threshold the value of 0.575. Comparing these values 0.575, 0.579 and 0.575 for the chloride threshold after glucose with that obtained from uninjected rabbits, i.e., 0.574 it is clear that glucose as such has no effect on the chloride threshold.

TABLE 2

NUMBER OF EXPERIMENT	INJECTION OF 4 PER CENT Na_2SO_4, $12H_2O$	THRESHOLD	URINE VOLUME AROUND THRESHOLD
	cc.		*cc. per minute per kidney*
1	80	0.624	2.4
2	143	0.600	1.0
3	153	0.636	1.0
4	167	0.673	0.6
5	200	0.722	0.3
6	229	0.626	3.0
7	279	0.675	1.2

Units as in table 1.

Sulphate: The effect of sulphate injections was examined in a similar fashion to that of glucose. Four per cent crystalline sodium sulphate (Na_2SO_4, 12 H_2O) solution was injected intravenously and subsequently 2 per cent sodium chloride in small quantities at a time, samples of blood and urine being taken in the intervals. Table 2 gives for 7 rabbits a determination of the threshold where curves could be drawn through the individual observations to cut the line of $C_u = C_B$. The sulphate injections in experiments 6 and 7 were not given all at once but in a few separate injections over a fairly long period. With four other rabbits the chloride injection after the sulphate was not pushed sufficiently far so that a curve for the threshold could be drawn but all of these would have considerably raised thresholds. If we take all observations with C_B within the normal range we get as average figures for 20 sets of observations on 11 rabbits $C_B = 0.599$, $C_u = 0.356$, and the urine volume per kidney = 0.540 cc. per min. per 2 kilos (urine volumes for the rabbit are expressed in these units

throughout the paper). From this it will again appear that the threshold must lie well beyond 0.600. Employing the equation form for all observations with $C_B > 0.600$, the equation for 25 observations is

$$C_u = 3.08\ C_B - 1.416 \qquad 5$$

from which $T = 0.681$. Here the mean value of C_u is 0.649, of $C_B = 0.670$ and of $V = 0.930$. Thus we may say that the chloride threshold after an average injection of approximately 180 cc. of 4 per cent sulphate lies in the region 0.650 – 0.685, i.e., a considerable increase in the normal threshold has taken place.

Urea: The effect of urea on the chloride threshold was determined in a similar fashion—injections of 5 per cent urea in 0.6 per cent saline being given and then small repeated injections of 2 per cent saline, urine and blood samples being taken as before. The effect was demonstrated on 5 rabbits with results as given in table 3 where the threshold for each rabbit

TABLE 3

NUMBER OF EXPERIMENT	INJECTION OF 5 PER CENT UREA IN 0.6 NaCl	THRESHOLD	URINE VOLUME AROUND THRESHOLD
	cc.		*cc. per minute per kidney*
1	81	0.707	1.4
2	89	0.644	0.4
3	90	0.595	0.6
4	143	0.682	0.8
5	87	0.650	0.5

Units as in previous tables.

was determined by a curve through the C_u concentrations plotted against C_B. Using an equation for all values of $C_B > 0.600$ we get

$$C_u = 4.10\ C_B - 2.101 \qquad 6$$

whence $T = 0.678$. Here the mean value of $C_u = 0.711$, of $C_B = 0.686$ and of urine volume = 0.863 cc. per minute per kidney, there being 18 sets of observations on the 5 rabbits. Hence we may conclude that the chloride threshold after approximately 100 cc. of 5 per cent urea injection is in the region 0.65–0.68, which like the effect of sulphate injections represents a considerable increase over the normal threshold.

IV. The absorption of chloride. As the injection of glucose in large quantities of 5 per cent strength has no effect on the chloride threshold, we have largely used it in producing variations in the plasma chloride and urine volume so as to examine how these factors influence the chloride absorption. From the "diffusion secretion" theory the urine is the same in volume as the glomerular fluid hence the ratio of the total amount of

chloride absorbed from unit volume of urine to the amount originally present is given by the ratio $\frac{C^{B}-C_u}{C_B}$. It is this ratio we have employed as the most suitable expression to find the effect of $t\left(\text{or } \frac{1}{V}\right)$ and of the

TABLE 4

AVERAGE URINE VOLUME	AVERAGE K_φ	AVERAGE K_φ (CORRECTED)	STANDARD DEVIATION OF K_φ	NUMBER OF OBSERVATIONS
cc. per minute per kidney				
0.151	0.161	0.161	0.039	12
0.349	0.156	0.164	0.035	12
0.493	0.169	0.175	0.039	12
0.622	0.166	0.169	0.035	12
0.774	0.162	0.163	0.037	12
0.881	0.169	0.171	0.036	12
1.094	0.164	0.169	0.034	12
1.694	0.176	0.178	0.038	12

$$K_\varphi = \frac{\sqrt{V} \times A}{\sqrt{(T - C_B) \times 100}}$$

In this expression

$$A = \frac{C_B - C_u}{C_B}$$

T = the threshold concentration of plasma. Concentrations are expressed as per cent NaCl. The table gives the average values of the observations ranged in order of urine volumes.

TABLE 5

AVERAGE $(T - C_B) \times 100$	AVERAGE K_φ	AVERAGE K_φ (CORRECTED)	STANDARD DEVIATION OF K_φ	NUMBER OF OBSERVATIONS
1.41	0.153	0.172	0.041	12
3.43	0.165	0.172	0.041	12
5.23	0.170	0.171	0.048	12
7.21	0.161	0.163	0.041	12
10.00	0.170	0.170	0.027	12
12.73	0.167	0.167	0.023	12
15.43	0.167	0.167	0.039	12
22.45	0.169	0.169	0.028	12

The table gives average values of the observations arranged in order of $(T - C_B) \times 100$. Units as in previous table.

plasma concentration C_B upon the chloride absorption. For brevity we shall call this absorption ratio A. As after a certain time it will be approximately unity, further increase of the absorption time or $\frac{1}{V}$ having then no

appreciable effect, it will be necessary to assign some limit to A which will be readily distinguishable from unity in the analysis of small quantities of urine; also, from the conception of an equilibrium towards which the diffusion tends, it must be supposed that the urine cannot be actually zero concentration. For these reasons we have taken an upper limit of A as 0.95 (which for C_u, is about 0.025). The equations arrived at from the analysis of 96 sets of observations from 20 rabbits of C_u, C_B (C_B being

TABLE 6 and 7

RANGE OF A	AVERAGE A	$\frac{T - C_B}{V} \times 100$	NUMBER OF OBSERVATIONS
0.0–0.2	0.119	2.7	7
0.2–0.4	0.322	5.8	18
0.4–0.6	0.505	9.7	35
0.6–0.8	0.706	17.7	36
0.8–1.0	0.855	26.3	21
RANGE OF $\frac{T - C_B}{V} \times 100$	AVERAGE $\frac{T - C_B}{V} \times 100$	AVERAGE A	NUMBER OF OBSERVATIONS
0– 5	3.31	0.256	16
5–10	7.23	0.489	34
10–15	12.60	0.589	20
15–20	17.30	0.713	23
20–25	21.50	0.725	5
25–30	27.40	0.769	11
30–35	32.70	0.878	4

Units as in previous tables.

under the threshold) may be stated as follows. Where the plasma concentration is constant and the volume varies we have

$$A = \sqrt{t} \times K_7 \qquad 7$$

(t being the time of unit volume of urine). Where the volume is constant, and the plasma varies we have

$$A = \sqrt{T - C_B} \times K_8 \qquad 8$$

T being the threshold. These may be combined in the general equation

$$A = \sqrt{t\,(T - C_B)} \times K_9 \qquad 9$$

This equation expresses the absorption for a range of A above 0.25, at lower values a correction to the formula must be introduced from the curve of A and $\frac{1}{t}$ (see fig. 1). Tables 4 and 5 show the independence of

K_9 in regard to changes in V or in $(T - C_B)$, the values of the 96 sets of observations after glucose injections being arranged in increasing order of V and $(T - C_B)$ respectively. For convenience the values of $(T - C_B)$ are given as $(T - C_B) \times 100$, the values of K_9 being also calculated from the latter unit, T and C_B being given as percentages. In the third column corrections have been introduced for the small number of very low values of A alluded to above. The value of K_9 may be stated as 0.169 ± 0.025. After sulphate injections similar results were obtained, the average value of K_9 as determined from 21 determinations being 164. Where corrections have been introduced for values of A below 0.250 this becomes 170. These determinations of K_9 were obtained from experiments where after a single injection of sulphate the chloride threshold was determined in the usual manner by subsequent injections of sodium chloride. Comparing the values of K_9 after sulphate, i.e., 164, (or 170 corrected) with those after glucose, 165, (or 169 corrected) it is evident that although sulphate has a marked effect on the chloride threshold, as previously shown, it does not otherwise influence the chloride absorption. It can be readily shown that varying the glucose injection and consequently its plasma concentration to the widest extent causes no appreciable change in K_9. For example, this will appear if we take the average K_9 for the first 10 values and for the last 10 of the 96 determinations ranged in order of increasing amount of injection. We get 0.164 as the average at the beginning and 0.172 as the average at the end of the series, these values being associated with average injections of 32 cc. and 302 cc. of 5 per cent glucose. From this it will appear that increasing the glucose injection about 10 times causes an increase in K_9 of only 5 per cent which lies within the error limit for the number of observations used. This result is remarkable in showing that the absorption of chloride is independent of the blood concentration of glucose (or sulphate) and is affected by the injected substance only in so far as this may alter the urine volume, the plasma chloride, and as in the case of sulphate, etc., the chloride threshold. Figure 1 gives the result of plotting the results of tables 6 and 7, i.e., the means of fixed ranges of $\frac{T - C_B}{V}$ against the means of A, and vice versa, for the figures obtained after sulphate and glucose injections as above considered. The actual curve drawn is a diffusion curve of t against the absorption ratio where iodine diffuses from chloroform into a stream of potassium iodide solution, the unit of time being chosen so that when the iodine is half absorbed, $t = 9.0$. (See below.)

The figures obtained after urea injections suitable for the calculation of the absorption constant were too few in number to illustrate the foregoing point; but such as were obtained fall within the distribution region after glucose or sulphate injections. We shall now consider somewhat

stricter statistical methods for the illustration of the absorption equation. Instead of taking limits for the dependent variable, A, it is better to assign corresponding limits to the independent variable $\frac{(T - C_B) \times 100}{V}$. These limits we have taken as 2.2 and 35 corresponding to $A = 0.25$ and 1.00, $\sqrt{\frac{V}{(T - C_B) \times 100}} \times A$ being 0.169.

TABLE 8

MEANS	STANDARD DEVIATIONS	CORRELATIONS OF ZERO ORDER	CORRELATIONS OF FIRST ORDER	REGRESSION COEFFICIENTS
$x_1 = 0.731$	$\sigma_1 = 0.183$	$r_{12} = -0.198$	$r_{12.3} = -0.70 \pm 0.03$	$b_{12.3} = -0.49 \pm 0.03$
$x_2 = 1.828$	$\sigma_2 = 0.300$	$r_{13} = +0.460$	$r_{13.2} = +0.76 \pm 0.03$	$b_{13.2} = +0.50 \pm 0.03$
$x_3 = 0.876$	$\sigma_3 = 0.353$	$r_{23} = +0.623$	$r_{23.1} = +0.82 \pm 0.02$	

$x_1 = \text{Log } (A \times 10)$
$x_2 = \text{Log } (V \times 100)$
$x_3 = \text{Log } \{(T - C_B) \times 100\}$

Number of observation sets = 117.
Units of V, C_B etc. as in previous tables.

Taking these limits of the independent variable we have 351 figures or 3 sets of 117 values of A, V and $(T - C_B)$ for statistical examination. If we consider equation 9 we can write it in the form

$$\text{Log } A = a \log V + b \log (T - C_B) + c \qquad 10$$

which will give the general form of all such equations as

$$A^n \times V^m \times (T - C_B)^p = K_{11} \qquad 11$$

In this formula log A is to be regarded as a variable dependent on log V and log $(T - C_B)$. It is required statistically to show the correlations between the three variables and to determine the best values of the coefficients a and b from the principle of least squares. These values of a and b should result as -0.5 and $+0.5$.

Table 8 gives a summary of the chief statistical data and the coefficients a and b are obtained as -0.49 and $+0.50$ the equation being

$$\text{Log } A = -0.49 \log V + 0.50 \log (T - C_B) + 0.209 \qquad 12$$

when $(T - C_B)$ is constant

$$\text{Log } A = -0.49 \log V + K_{13} \qquad 13$$

shows the influence of volume on absorption. This is practically the same as

$$A = \sqrt{\frac{1}{V}}\, K_{14} \qquad 14$$

or

$$A = \sqrt{t}\, K_{14}$$

where V is constant, then

$$\text{Log } A = 0.50 \text{ Log } (T - C_B) + K_{15} \qquad 15$$

or

$$A = \sqrt{T - C_B} \times K_{16} \qquad 16$$

TABLE 9

$(T - C_B) \times 100$	$V_{(A=0.5)}$	$\frac{V_{(A=0.5)}}{(T - C_B) \times 100}$	C_B	NUMBER OF OBSERVATIONS	NUMBER OF RABBITS USED
1.41	0.151	0.107	0.559	12	9
3.43	0.413	0.120	0.544	12	10
5.23	0.634	0.121	0.522	12	8
7.21	0.795	0.110	0.508	12	9
9.97	1.192	0.119	0.479	12	9
12.73	1.448	0.114	0.447	12	8
15.43	1.812	0.117	0.422	12	9
22.49	2.597	0.115	0.349	12	8

The table gives average values for the observations arranged in order of $(T - C_B) \times 100$. Units as before.

Another way of illustrating equation 9 is to show K_9 by statistical means independent of V and $(T - C_B)$.

The correlation of K_9 with V for 117 sets of observations between the limits already mentioned is $+0.047 \pm 0.06$ which is equivalent to complete independence. The volume effect may be shown in the equation

$$K_9 = + 0.0037\ V + 0.164 \qquad 17$$

where a change of V from 0.1 to 2.0 cc. per minute causes only a 4 per cent change in K_9.

The correlation of $(T - C_B)$ with K_9 is 0.00 ± 0.06 so that K_9 is also completely independent of changes in $(T - C_B)$ though these changes are of very wide extent.

Using the formula $A = \sqrt{t}\, K_{14}$ at constant $(T - C_B)$ we can for each observation of V and A calculate the value of V at which A is equal to 0.5. Table 9 gives a summary of the figures obtained in this way after glucose injections. It will be seen that the average urine volumes required for half absorption of the chloride (i.e., $C_u = \frac{1}{2}\, C_B$) are directly proportional to $(T - C_B)$. In other words, from the diffusion theory, the rate of chloride diffusion through the cells is proportional to the distance of the plasma concentration below the threshold. This is one of the most important results obtained from the development of the theory. In column 4 of the table are also given the average corresponding plasma

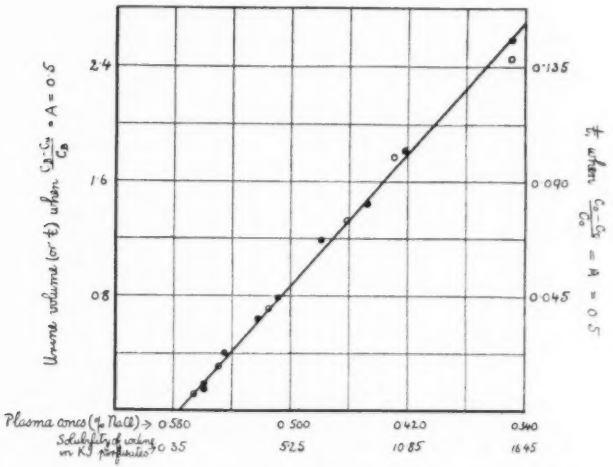

Fig. 2. Dots represent plasma concentrations. Circles represent iodine solubilities. Figures from tables 9 and 11.

concentrations. Figure 2 shows the results of plotting the average values of half absorption in table 9 against the average plasma concentrations. The line through the points cuts the abscissa at 0.575. The correlation coefficient between the logs of the volumes of half absorption for all the observations and the logs of $(T - C_B)$ values is $+0.86 \pm 0.02$. The statistical equation of least error in calculating log V from log $(T - C_B)$ is

$$\text{Log } V = 0.93 \log (T - C_B) + 0.977 \qquad 18$$

This equation also shows the linear relation between the volumes of $\frac{1}{2}$ absorption and $(T - C_B)$. (The linearity of the regression itself may be shown by the use of the correlation ratio.)

As already mentioned the volumes of $\frac{1}{2}$ absorption have been calculated for each V and A value from the relation $A = \sqrt{t}\, K_{14}$. As this equation can be established independently of a value being assigned to the threshold, figure 2 amounts to an independent determination of the chloride threshold. Stating this result in more general terms, we may say that for urines where $C_u < C_B$, (C_B being reduced by glucose injection) plotting the average volumes where $C_u = \frac{C_B}{2}$ against C_B gives a strictly linear relation holding without apparent limits over the widest range obtained. The line obtained cuts the abscissa at 0.575. From the standpoint of the diffusion theory

TABLE 10

A	TIME VALUES (t) FOR THE DIFFERENT PERFUSATES THE UNIT OF TIME BEING TAKEN FOR EACH WHEN $A = 0.5$					$\sqrt{\frac{1}{t_1}} \times A$
	General curve: the average perfusate being 9 per cent potassium iodide	20 per cent potassium iodide	13 per cent potassium iodide	5 per cent potassium iodide	1 per cent potassium iodide	
0.1	0.10	0.10	0 11	0.08	0.09	0.32
0.2	0.24	0.25	0.25	0.20	0.25	0.41
0.3	0.43	0.45	0.43	0.40	0.44	0.46
0.4	0.67	0.68	0.68	0.67	0.66	0.49
0.5	1.00	1.00	1.00	1.00	1.00	0.50
0.6	1.40	1.46	1.38	1.43		0.50
0.7	1.94		1.92	1.94		0.50
0.8	2.70		2.66	2.70		0.49
0.9	3.75		3.95			0.46

Depth of chloroform layer = 8.0 mm.
Fixed plunger at 2 mm. from surface of chloroform.
Perfusion rate = a cc. per minute.
Average temperature = 15.4°.
(For general curve, col. 2; see figure 1: the unit of time in the figure being nine times that in the table.)

this result is readily explained by the conception that the diffusion rate of chloride through the cells is directly proportional to the distance of the plasma from the threshold. This result has a striking analogy with diffusion in vitro across surfaces of varying partition coefficient, as we shall see in the next section.

IV. DIFFUSION ANALOGY TO THE CHLORIDE ABSORPTION. The diffusion of iodine from chloroform into a stream of potassium iodide solution was selected for investigation, as the partition coefficient can be varied over a wide range. The technique employed was similar to that described in a previous paper (Conway and Kane, 1926) where the diffusion of iodine from water into chloroform was examined.

The potassium iodide solution was introduced over the surface of some solution of iodine in chloroform of measured depth in one fixed cylinder of the Kober colorimeter. The perfusing solution passed down a capillary tube which opened under the plunger (arranged as previously described) at 2 mm. from the surface of the chloroform. The perfusing fluid was fed at a constant rate of about 1 cc. per minute and the overflow drained by capillary siphons. The total concentration of iodine in the chloroform of the fixed cylinder could be obtained at any time by comparing with standard solutions in the adjustable cylinder. Table 10 gives figures obtained from the absorption curves on perfusing with various strengths of potassium iodide. Arbitrary units of time are chosen for each iodide concentration

TABLE 11

PERFUSION FLUID	PERIOD OF ½ ABSORPTION	NUMBER OF EXPERIMENTS	SOLUBILITY OF IODINE IN PERFUSATE	AVERAGE TEMPERATURE	SOLUBILITY × ½ VALUE PERIODS
per cent potassium iodide	*minutes*		*grams per liter*		
20	7.2	2	15.86	15.8	114
13	10.0	2	10.37	15.2	104
10	13.4	2	8.03	15.3	107
5	25.0	1	4.18	16.9	105
2	55.2	4	1.87	15.7	103
1	100.5	3	1.11	15.9	111
0.5	150.5	2	0.73	15.4	110

In all experiments of this table there was a perfusion rate of 50 to 70 cc. per hour and a temperature of 15.0–17.0°C.

so that the time of half absorption is in each case represented by unity. A represents as in part III the absorption ratio or $\frac{C_o - C_{t_1}}{C_o}$, C_o and C_{t_1} being the mean concentrations after times o and t_1. As can be seen from the table, variations in the concentration of potassium iodide have no effect on the form of the curve although the actual diffusion rate of the iodine increases 14 times from a perfusion with 1 per cent iodide to 20 per cent iodide as will appear from table 11. As will be seen from table 9, the curve of A and t can be approximately characterized at values of $A > 0.25$ by the relation

$$A = \sqrt{t_1} \times K_{19} \qquad 19$$

Attention must be paid in these perfusion experiments to secure uniform conditions of temperature, absence of vibrations, as well as of cloud formation, air bubbles, dust etc., at the interface.

In examining the influence on the diffusion rate of changes in the perfusing fluid we have taken for comparison the half absorption period.

Table 11 gives the half-absorption periods for the various strengths of iodide perfusion. In the table is given the solubility of iodine in the potassium iodide solutions as obtained from the figures given by Mellor (1922). These are values obtained at 0°C. but it is taken that the relative solubility will be little altered at 15°C. It will be seen that the diffusion rate of the iodine is directly proportional to its solubility in the perfusing fluid, this linear relation holding over a very wide range of solubility. This is also illustrated in figure 2 from the figures of table 11. When A is fixed we have then the equation

$$S \times t_1 = K_{20} \qquad 20$$

S being the solubility of iodine in the perfusing fluid. Combining equations 19 and 20 we obtain

$$A = \sqrt{t_1 \times S} \times K_{21} \qquad 21$$

or

$$\text{Log } A = + 0.50 \log t_1 + 0.50 \log S + K_{22} \qquad 22$$

which we may compare with the equation for chloride absorption, i.e.,

$$\text{Log } A = + 0.51 \log t + 0.50 \log (T - C_B) + K_{12} \qquad 12$$

t in this case being time of unit volume of urine. In the chloride absorption equation the value of $(T - C_B)$ enters analogously to the solubility of the iodine in the perfusing fluid of the model diffusion experiments, that is, to the partition coefficient between the chloroform and the perfusing fluid.

This striking analogy is in strong support of the idea already suggested in a previous paper that it is upon an alteration of solubility relations that the function of secretion depends, whether this secretion is into the urine or from the urine or cell into the blood.

V. In this and the preceding communications sufficient evidence has been presented to show that the concentration and volume relations of secretion and absorption are expressed by the relations.

$$\frac{C_u}{C_B} = \sqrt{t}\, K_A \qquad 23$$

$$\frac{C_u - C_B}{C_{\text{max.}} - C_B} = \sqrt{t}\, K_B \times \sqrt{D} \qquad 24$$

or

$$\Sigma \frac{C_u - C_B}{(C_{\text{max.}} - C_B)\sqrt{D}} = \sqrt{t}\, K_B$$

$$\frac{C_B - C_u}{C_B} = \sqrt{t}\, K_C \times \sqrt{T - C_B} \qquad 9$$

Equation 23 expresses the excretion of urea, in the human subject at the normal blood range. Evidence has been presented in previous paper that it also expresses the excretion of sulphate and phosphate for the human subject (subject to the same upper volume limit). It would seem therefore to be a general relation for the lower blood concentrations—not considering substances with high thresholds or those manufactured by the tubule cells.

Equation 24 holds as a general relation when the blood is much raised by injections. Equation 9 is the chloride absorption equation developed in this paper.

TABLE 12

CONSTANT	CORRELATION COEFFICIENT WITH URINE VOLUME	CORRELATION COEFFICIENT WITH PLASMA CONCENTRATION	THEORETICAL CORRELATION WITH PROBABLE ERROR FOR A NORMAL DISTRIBUTION	COEFFICIENT OF VARIATION OF K_A, K_B AND K_C	NUMBER OF OBSERVATION SETS
Log K_A	−0.025	−0.052	zero ±0.05	25	177
K_B	+0.073	+0.062	zero ±0.08	22	75
K_C	+0.047	+0.000	zero ±0.06	26	117

The correlations of Log K_A were obtained with the logs of the urine volume and plasma concentration. For K_A the expression $\frac{V^{0.51} C_u}{C_B{}^{0.56} W^{0.32}}$ was used. (See section IV of previous communication.)

These three equations are independent of theory but their diffusion analogies are so striking that an explanation based on diffusion seems inevitable, particularly, as since the second has been shown to be a general equation and the value of K_B inversely proportional to the square root of the respective diffusion coefficients. From the diffusion standpoint the expressions to the left are the amount of substance diffused into (or from) unit volume of urine divided by the diffusion head in equation 24, by C_B which is proportional to the diffusion head in 23, and to the diffusion head at beginning of absorption in equation 9.

The three constants in the equations are independent of the independent variables urine volume and plasma concentration. The correlations showing this independence are collected in table 12 and it may be noted that all the figures for determining K_A and the large majority for K_B have been taken from the work of independent observers. The table also shows that the coefficient of variation is practically the same in the three. A much smaller variation is obtained for these constants if we confine ourselves

to the individual subject under equable conditions as shown in the paper of Austin etc. (1921) also in (Conway and Kane, 1926; Conway, 1925). The independence of the constants in regard to urine volume and blood concentration made it a matter of interest to plot the curve of their frequency distribution to see if the existence of other influences would be manifested by some characteristic distribution.

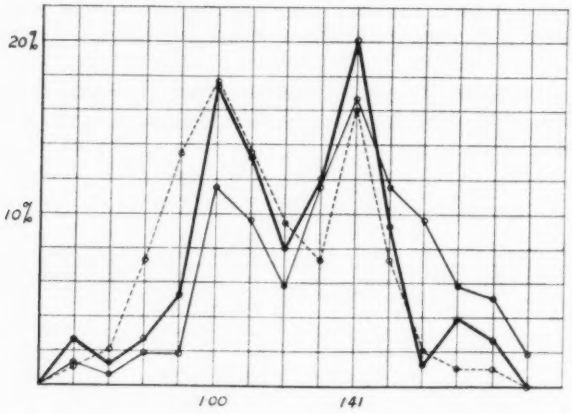

Fig. 3. The thin line represents K_A, thick line K_B and the dotted line K_C, the class intervals for the three being 0.300, 0.020 and 0.015 K_A and K_B are the K_6 and K_8 of previous communication with units as used there in tables 12 and 10. K_C is the K_9 of present paper with units as in table 5. The point 1.00 on the abscissa is the mid-point of the intervals 2.80–3.10, 0.185–0.205 and 0.140–0.155 for the three constants.

For K_A we have taken 156 values from the figures of the following observers

Addis and Watanabe (1916), (70 values, 32 subjects)
Walker and Rowe (1927a), (47 values, 12 subjects)
McLean (1915), (39 values, 19 subjects)

We have neglected the values for any individual subject for whom more than 10 sets of figures are recorded as they could be considered as unduly influencing the distribution in the series. This meant the exclusion of the personal figures of Addis and McLean, otherwise apart from the usual volume limit no principle of selection was adopted. For K_B we have the 75 figures as commented on in previous communication, section III, and for K_C the 96 values after glucose injections as mentioned in the present paper. The result has been a curious one as will be seen from figure 3. Whilst larger numbers of results than those used would be in general

advisable for such frequency curves yet the fact that for the three curves there is the same uniform distribution around two peaks, which have in each case the ratio 1:1.41 is a result that may not be assigned to a chance distribution. The ratio can also be expressed as $1:\sqrt{2}$ and the distribution find an explanation in a tendency for the amount of active tissue to be doubled in certain circumstances.

Whatever be the explanation, the fact of so curious a similarity of distribution with different substances and processes is another point in the evidence that the constants in question are a true indication of the renal activity.

VI. The relation of the theory here advocated to other lines of research may now be considered.

The facts so far recorded from the direct examination of the glomerular fluid (Wearn and Richards, 1925) and the cannulization of the tubules of the Necturus (White and Schmitt, 1926) are in harmony with the diffusion theory which supposes a glomular fluid of practically the same concentration as a filtrate except in the case of chloride. The exceptional position of chloride is here in line with the exceptional position of chloride in applying the secretion formula, though as before mentioned after large injections of glucose, sulphate, etc., we have found it better to suppose that the glomerular fluid was identical with a blood filtrate. The evidence presented by these workers as to the general fact of chloride absorption is also in harmony with the views here presented. We may say that no convincing evidence has been given for the absorption of water in the frog. Comparisons of the amount of glomerular fluid obtained and the amount of urine secreted do not seem of consequence unless the same resistance to flow exists in both cases. Some evidence of water absorption presented by Hayman and Richards (1926), such as the formation of precipitates of indigo carmine and sodium carminate injected into the tubule lumen are susceptible of another explanation than that afforded by water absorption. For example, indigo carmine in the strength injected was found to be readily precipitated by a sample of normal human urine and a precipitate was found on standing even when the urine was diluted four times. It may be pointed out that from the standpoint of the diffusion theory the film of fluid along the wall of the tubules can be very much greater in concentration than the bladder urine. It may indeed also be added that this particular line of evidence is not stressed by these workers. Concerning water absorption in the frog there is a striking experiment of Clark's (1922) showing that though glucose is absorbed by the tubules water is not absorbed, a conclusion in accordance with the theory advanced here.

In Clark's experiments "the renal arteries were perfused with a fluid containing less than 0.1 per cent glucose, while the venous perfusion

contained 0.4 per cent or 0.5 per cent." The venous perfusion took place through the renal portal. When the mixed perfusate in the tubule capillaries (as collected from the vena cava) was less than 0.21 per cent the urine concentration was less than the arterial perfusate. When the mixed perfusate was greater than 0.21 per cent the urine had a glucose concentration identical (within the error limits) with the arterial perfusate. This identical relation was maintained even when the mixed perfusate was ten times as concentrated as the arterial perfusate.

Two conceivable explanations from the water absorption point of view may be advanced. One, that the kidney became inactive when the mixed perfusate exceeded 0.21 per cent glucose, the other that glucose and water were absorbed in exactly the same concentration as the glomerular fluid. Both of these explanations are highly improbable from the water absorption theory. The kidney must either have been active since it maintained a marked difference of concentration on either side of the cell "the physical conditions inviting the passage of water from the glomerular filtrate to the more concentrated fluid in the tubule capillaries" or else in becoming inactive the cell became at the same time impermeable both to water and to glucose. In support of this suggestion that the kidney became inactive, Bieter and Hirschfelder (1924) suggest that similar results were obtained by Clark after poisoning with mercuric chloride. But in this connection Clark states "*both* aorta and renal portal vein were perfused with fluid containing less than 0.1 per cent glucose" and "In every case the urine contained the same percentage of glucose as *the* perfusing fluid." (The italics are ours. Compare with perfusion of unpoisoned cells quoted above.) From this and the context, it may, we think, be taken for granted that the arterial and venous perfusates were of the same concentration and the result obtained the most likely one after the tubule poisoning.

As for the explanation that a solution of the same glucose concentration as the glomerular fluid was absorbed it may be said that on the water absorption view the absorption rates of water and glucose into the blood are not in general equal (see section I) and are very unlikely to become and remain so when the glucose concentration in the tubule capillaries is indefinitely increased. In short, the only likely explanation is the one drawn by Clark, that though glucose may or may not be absorbed, according to the conditions, water is not absorbed. That a mixing of arterial and renal perfusates could have occurred in the glomerular vessels—a question here arising from recent work of Richards and Walker (1927) seems impossible from the identical composition of the arterial perfusate and the ureteral fluid.

The study of renal secretion by dye injections has received a recent extension in the intracapsular injection of dyes by Hayman and Richards (1926). The main fact that such dyes can diffuse from lumen into cell is

in accordance with the diffusion theory which assumes a balance or diffusion to a balance of concentrations in cell and lumen. The theory is also in complete harmony with the strong evidence brought by Richards and Walker (1927) that all the fluid of the urine in the frog comes from the glomerulus.

Another line of research has been concerned with the investigation of concentration ratios. According to the theory of Cushny (1926) there must be an equal degree of concentration of all non-threshold substances during their passage through the tubules. Mayrs (1922) studied from this standpoint the concentration ratios of creatinine, sulphate, phosphate and urea in rabbits after injections. He found the first three concentrated to the same extent whereas urea was concentrated less. The comparison with sulphate and urea was made however almost entirely at normal urea plasma concentrations and very much increased sulphate. In a subsequent paper (1923) where urea was also injected as well as sulphate the concentration ratio advances to about the same figure. It may be concluded from Mayrs' work that in general where the blood concentration is much raised by injections (chloride being excepted) the concentration ratio of the different substances tends to the same value, whereas if the blood concentration is not so increased by injections, exceptions tend to be multiplied by more extended research, e.g., White (1923a, 1923b, 1923c), Rehberg (1926). In a previous communication (section III) it was shown that where two substances were injected we have the relation

$$\frac{\sqrt{\frac{V}{D}}\,(C_u - C_B)}{\sqrt{\frac{V}{D_1}}\,(C_{u1} - C_{B_1})} = \frac{C_B}{C_{B_1}}$$

i.e.

$$\sqrt{\frac{D_1}{D}} \times \frac{C_u - C_B}{C_{u1} - C_{B_1}} = \frac{C_B}{C_{B_1}}$$

where D does not much differ from D_1. $\sqrt{\frac{D_1}{D}}$ may be eliminated, i.e.

$$\frac{C_u - C_B}{C_{u1} - C_{B_1}} = \frac{C_B}{C_{B_1}},$$

from which

$$\frac{C_u}{C_B} = \frac{C_{u1}}{C_{B_1}},$$

or the concentration ratios are equal. The significance of the relation

$$\frac{\sqrt{\frac{V}{D}}(C_u - C_B)}{\sqrt{\frac{V}{D_1}}(C_{u1} - C_B)} = \frac{C_B}{C_{B_1}}$$

from which the equality of the concentration ratios is deduced arises from the fact that with the diffusion theory the ratio on the left must also express the ratio of the diffusion heads of the two solutes (see previous communication, section I). That is to say, the sum of the diffusion heads remaining constant at high blood values (see section III of same reference), their ratio is then proportional to the plasma concentrations, a result which would be in agreement with the theory presented, though not necessarily demanded by it. We may finally mention the relation of the theory to the effect of temperature upon renal secretion. It was shown empirically by one of us in a previous communication (Conway, 1925) that temperature had the same effect on the value $(C_{max} - C_B)$ for glucose, urea and chloride, the chloride value being taken as $(C_{max} - 1.25\ C_B)$.

This result receives now its theoretical explanation. The expression $(C_{max} - C_B)$ as there used is approximately 75 per cent of the diffusion head (see section 1, previous communication) and the effect of temperature is shown therefore to act on the diffusion head being the same effect for the three substances.

In conclusion we may say that the theory advanced is in harmony with the main pertinent facts of renal physiology and is capable of explaining them by relations based on simple diffusion processes and a vital state of the active kidney cells which whatever its real nature may be, can be adequately represented as to time and concentration relations by a simple physical parallel.

SUMMARY

The chloride threshold is defined as the highest value or limit of C_B (the plasma concentration) at which C_u (the urine concentration) is $< C_B$ for all rates of urine flow. The chloride threshold for the rabbit (under urethane anesthesia) is 0.574 per cent agreeing with that obtained for the human subject using the figures of McLean (1915). The injection of glucose has no effect on this chloride threshold even with injections of over 300 cc., 5 per cent. On the other hand the injection of sulphate or urea (in saline) causes a marked increase in the chloride threshold.

From the premises of the diffusion-secretion theory, that is, assuming the absorption ratio for a given volume of urine to be expressed as $\frac{C_B - C_u}{C_B}$

it is shown that the rate of absorption of sodium chloride is directly proportional to $(T - C_B)$ where T is the threshold, also that, at constant C_B the absorption ratio is proportional to $\sqrt{t}\left(\text{or } \frac{1}{\sqrt{v}}\right)$ where t is the time of unit volume of urine. These relationships are shown to have a close analogy in the diffusion of iodine from chloroform into potassium iodide solutions.

The diffusion secretion theory presented in this and previous papers is shown to be in harmony with the facts established in other lines of renal research.

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THE CROSSED EXTENSOR REFLEX IN DEAFFERENTED MUSCLE

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In a recent study of the rôle of the dorsal roots in muscle tonus (Ranson, 1928) our attention was drawn to the crossed extensor reflex. We found that this reflex could be easily obtained from a deafferented leg in an otherwise intact and unanesthetized cat. The response was steady and persistent and in many instances it appeared to outlive the stimulus by an appreciable amount. It imposed on the limb a definite posture and appeared to be as truly tonic as are the labyrinthine and neck reflexes described by Magnus (1924). Since the crossed extensor reflex in deafferented muscle has been said to be atonic (Sherrington, 1909) it seemed desirable to study these apparently tonic responses in decerebrate cats.

In adult cats the dorsal roots of the right lumbosacral plexus were cut intradurally under anesthesia and with aseptic precautions. The operative technique, which is a matter of considerable importance, has been described in connection with some experiments on the relation of the dorsal roots to muscle tonus (Ranson, 1928). In all but two instances the dorsal roots of the right lumbosacral plexus were cut from the sixth lumbar to the third sacral inclusive. In cat D-21, the L IV, V, VI, VII and S I and II were cut and in cat D-27, the L V, VI, VII and S I, II, and III. The cats stood the operation well and the wounds healed promptly and without infection. Decerebrations were performed several weeks later; the exact time between operation and decerebration in each instance is indicated in the tables.

THE CROSSED EXTENSOR REFLEX AFTER ANEMIC DECEREBRATION. Some of the decerebrations were made by the anemic method (Pollock and Davis, 1923), i.e., by ligation of the basilar and both carotid arteries. The animal was then prepared for stimulation of the left popliteal nerve and for recording the contractions from the right gastrocnemius. The muscle was laid bare and dissected free up to its two heads of origin from the femur, care being taken to avoid injuring the blood vessels and nerves which enter the muscle at its upper end. The femur was secured by a drill

passed through the condyles and by a clamp near its upper end. The tibia was drawn upward and held firmly with the knee in full extension by a string attached to the foot. The cat lay on its back with its front legs tied to the board, its left hind leg held vertically by a string attached to the foot (so that the popliteal nerve might be readily accessible), and the head secured by a bar passing through the mouth (to prevent rotation of the head). The position of the body and head are of importance in studying tonic reflexes because stimuli arising in the labyrinths, in the proprioceptors of the neck and in the exteroreceptors of the side of the body exert a

TABLE 1

Crossed extensor reflexes after anemic decerebration in normal and deafferented cat's gastrocnemius

Time in seconds

CAT NUMBER	DAYS AFTER OPERATION	STIMULUS		LATENT TIME	TIME FOR:	
		Coil	Duration		¼ relaxation	¾ relaxation
Normal						
N-2		12	0.25	0.04	1.04	
N-3		12	0.25	0.04	2.16	
N-4		12	0.25	0.04	3.24	
N-5		12	0.25	0.05	0.50	
N-6		12	0.25	0.04	2.10	
N-7		12	0.25	0.07	2.64	
N-8		12	0.25	0.04	1.68	
N-9		12	0.25	0.06	4.28	
Deafferented						
D-1	25	12	0.25	0.14	3.58	6.28
D-2	14	12	0.25	0.07	2.08	6.08
D-3	16	12	0.25	0.08	2.82	6.72
D-4	16	12	0.25	0.06	0.44	0.48
D-5	16	12	0.25	0.15	0.86	1.86
D-6	17	12	0.25	0.05	2.22	4.54
D-7	35	10	0.58	0.05	2.56	4.8+
D-8	35	12	3.58	0.08	17.4+	17.4+

profound influence on the state of the tonic centers in the brain stem (Magnus, 1924). The Achilles tendon was attached by heavy linen thread to an isotonic muscle lever having a ratio of 1:5. Traction was exerted by a rubber band instead of weights and this was adjusted so as to exert a tension of 500 grams on the muscle when the writing lever was horizontal. All tension was removed from the muscle during the resting periods. Time was recorded by a fork marking fiftieths of a second. Shielded electrodes were attached to the central end of the divided popliteal nerve. In the experiments on cats decerebrated by the anemic method the primary circuit of the Harvard inductorium was closed by a mechanical device for

periods of 0.25 second and the secondary coil was set at 12. The resistance in the primary circuit, due in part to the fine wire in the signal marker, was so great that two dry cells were required to operate it.

During the experiments care was taken to keep the body temperature of the cat normal and the gastrocnemius muscle was kept warm and moist by covering it with a pledget of cotton during the intervals between stimulations and allowing normal salt solution at 39°C., to run over it.

The results are given in table 1 along with those obtained from eight normal cats decerebrated in the same manner. The latent period, measured from the beginning of the stimulus to the beginning of the mechanical response, varied from 0.04 to 0.07 second for the normal muscle and from 0.05 to 0.15 second for the deafferented muscle. This seems to indicate a slight increase in the latent time as a result of previous section of the dorsal roots supplying the muscle. The latent time also seems to be more variable in muscles deprived of their sensory fibers.

In deafferented muscle the time required for the writing lever to fall $\frac{3}{4}$ of the distance from the high point of the contraction to the base line, i.e., the time required for $\frac{3}{4}$ relaxation, varied from 0.48 to more than 17 seconds. It should be noted that this slowest relaxation followed a prolonged stimulus lasting 3.58 seconds. Cat D-4 which gave the quickest relaxation never developed decerebrate rigidity in the hind limbs. If this experiment is excluded the quickest relaxation was in cat D-5, where 1.86 seconds after cessation of the stimulus elapsed before the lever fell $\frac{3}{4}$ of the distance to the base line. In all the other experiments 4.5 seconds or more were required for $\frac{3}{4}$ relaxation. In cats D-7 and D-8 the drum was

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Fig. 1. Crossed extensor reflex in the deafferented gastrocnemius of cat D-11 after transection of the brain stem at the upper border of the mesencephalon. Secondary coil at 10.

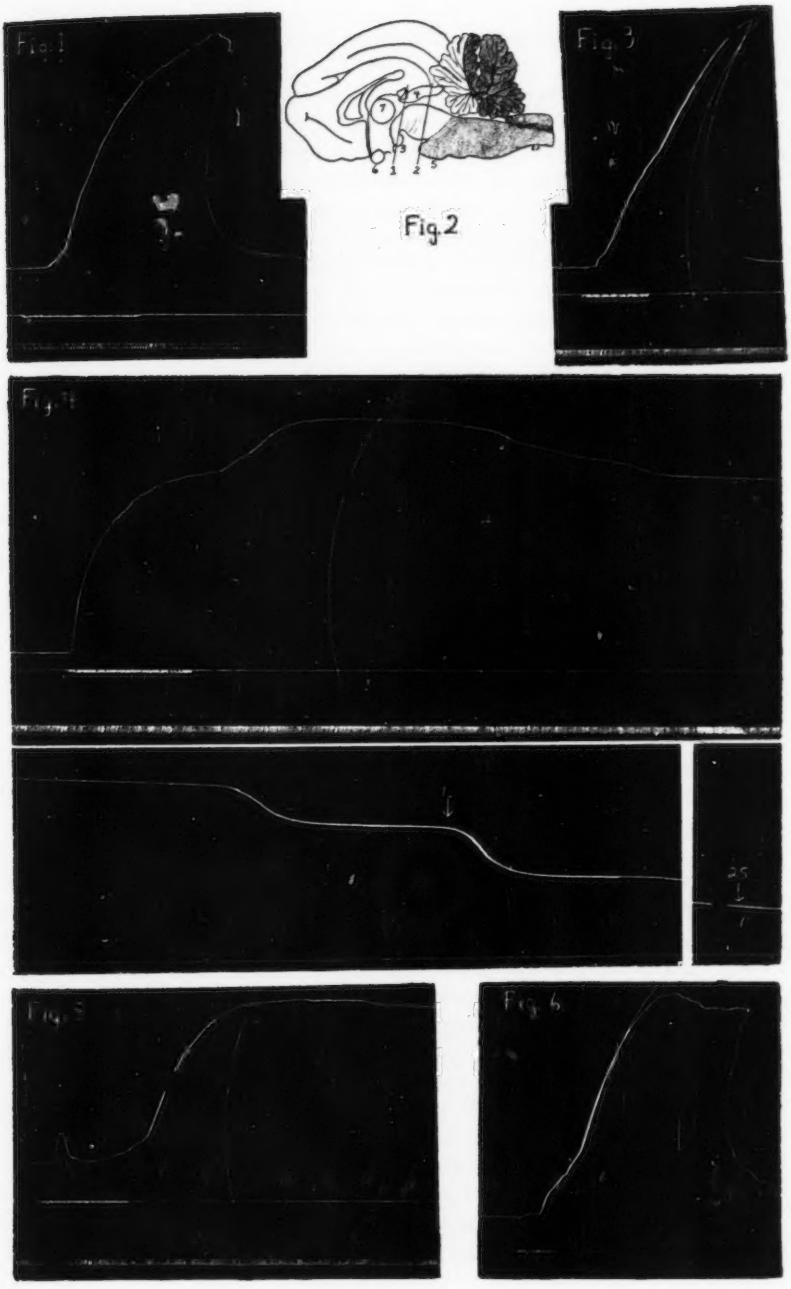
Fig. 2. Diagram of median sagittal section of the cat's brain. The parts not rendered anemic by the ligation of the carotids and basilar arteries are shaded. Line *1* represents the level of the high transections and line *2* the level of the low transections of the brain stem: *3*, mammillary body; *4*, lamina quadrigemina; *5*, pons; *6*, optic chiasma; *7*, massa intermedia.

Fig. 3. Crossed extensor reflex in the deafferented gastrocnemius of cat D-28 after transection of the brain stem at the upper border of the mesencephalon. Secondary coil at 17.

Fig. 4. Crossed extensor reflex in the deafferented gastrocnemius of cat D-28 after transection of the brain stem at the rostral border of the pons. Secondary coil at 16. Beginning of the reflex above and continuation below. The two arrows mark the lapse of 13 and 25 seconds respectively after the end of the stimulus.

Fig. 5. Atypical crossed extensor reflex in the deafferented gastrocnemius of cat D-8. Secondary coil at 12.

Fig. 6. Atypical crossed extensor reflex in the deafferented gastrocnemius of cat D-24 after high decerebration. Secondary coil at 10.



Figs. 1 to 6

stopped before this degree of relaxation was obtained. In the latter at the end of 17.4 seconds the relaxation was only $\frac{2}{3}$ completed.

In normal cats decerebrated by the same method the rate of relaxation may have been somewhat slower; but in all these experiments the drum was stopped before the relaxation was $\frac{3}{4}$ completed and for this reason a satisfactory comparison cannot be made. The table gives the time required for $\frac{1}{3}$ relaxation in both the normal and deafferented muscle. This varied from experiment to experiment but was not noticeably different in the two series except for the slow relaxation in cat D-8 which is probably to be accounted for by the prolonged stimulus. One thing stands out clearly, however. The rapid, almost instantaneous relaxation of the crossed extensor reflex obtained by Sherrington in deafferented muscle appeared but once in our records and that was in a cat which had failed to develop decerebrate rigidity in the hind legs.

In some of Sherrington's cats, decerebrated a few hours or a few days after dorsal root section, a brief after-discharge could be seen in the crossed extensor reflex sometimes lasting as much as 5 seconds, the duration depending on the strength of the stimuli. The description and figures indicate that in these instances the contraction was maintained for a few seconds by after-discharge and then abruptly subsided, the relaxation, when it set in, occurring very rapidly. This rapid subsidence differentiated these reflexes from those obtained in normal animals. We have obtained similar tracings from deafferented muscles after high decerebration (fig. 6). The contraction was maintained at its height for two or three seconds after cessation of the stimulus and then abruptly relaxed. Such responses were obtained only after high decerebration and with strong stimuli and differ fundamentally from the normal in the abruptness of the relaxation.

Since in our first six experiments only two or three weeks had elapsed between the operation and decerebration, it seemed possible that a longer period of recovery might lead to prompter relaxation. Sherrington (1909) never obtained the prolonged after-discharge in animals that had survived dorsal root section for more than 26 days. For this reason two of our cats (nos. D-7 and D-8) were allowed to survive the operation for 35 days. But in these experiments the relaxation of the muscle was even slower than in the preceding six. We felt sure therefore, that the period of recovery following the operation was not the determining factor and thought that the anemic decerebration might be responsible for the slow relaxation.

Cats decerebrated by the anemic method develop a higher grade of rigidity than do those with transection above the level of the tentorium. The extensor rigidity is so great that it is usually difficult to demonstrate plasticity. If the legs are passively flexed they tend to spring back at once into full extension again as soon as released.

THE CROSSED EXTENSOR REFLEX AFTER TRANSECTION OF THE BRAIN STEM. Since it seemed possible that the greater activity of the center for extensor tonus in the hind brain was responsible for the slow relaxation of deafferented muscle in the crossed extensor reflex after anemic decerebration, it was necessary to repeat the experiments on animals decerebrated in the usual manner. Ten cats in which the dorsal roots had been cut two to five weeks previously were employed, as well as four normal ones for controls.

Under ether anesthesia a tracheal cannula was inserted and the carotids tied. The calvarium was exposed by a transverse incision and opened with a drill and rongeur forceps over the occipital poles of both cerebral hemispheres. The dura was opened by a transverse incision after the superior sagittal sinus had been doubly ligated and cut between the ligatures. The cerebral hemispheres were raised and the brain stem divided at the level of the upper border of the superior colliculus. All of the brain in front of the cut was removed and the blood sponged away with cotton until hemorrhage ceased. The cranium was not packed with cotton nor were the vertebral arteries compressed at any time. Compression of these arteries deprives the medulla oblongata of blood, causes interference with respiration and is likely to produce shock which delays the onset of rigidity.

After the completion of the experiment the brain stems were removed, fixed in formalin and the exact level of the transection determined. The levels were found to be as follows:

Cat N-10. Just in front of the superior colliculus and just behind the mammillary bodies.

Cat N-11. No autopsy.

Cat N-13. Just in front of the left superior colliculus, and through the middle of the right superior colliculus and through the infundibulum.

Cat N-14. Just in front of the superior colliculus and through the infundibulum.

Cat D-10. Through rostral border of the superior colliculus and the mammillary bodies.

Cat D-11. Through rostral border of the superior colliculus and through the upper end of the cerebral peduncle.

Cat D-12. Through rostral third of the superior colliculus and just caudal to the mamillary bodies.

Cat D-15. Through rostral half of the superior colliculus and through the cerebral peduncles 3 or 4 millimeters rostral to the pons.

Cat D-17. Through middle of the inferior colliculus and just rostral to the pons.

Cat D-21. *High section.* Through rostral border of the superior colliculus and through the mammillary bodies. *Low section.* Between the superior and inferior colliculi and through the rostral border of the pons.

Cat D-24. *High section.* Through rostral border of the superior colliculus and just in front of the optic chiasma. *Low section.* Through the caudal part of the superior colliculus and the rostral border of the pons.

Cat D-27. *High section.* Through the rostral border of the superior colliculus and just in front of the mammillary bodies. *Low section.* Through caudal border of the superior colliculus and the rostral border of the pons.

Cat D-28. *High section.* Rostral border of the superior colliculus and upper end of cerebral peduncle. *Low section.* Between the superior and inferior colliculus and through the rostral border of the pons.

Cat D-30. Through rostral border of superior colliculus and just in front of the mammillary bodies.

TABLE 2

Crossed extensor reflexes in normal and deafferented cat's gastrocnemius after transection of the brain stem at different levels

Time in seconds

CAT NUMBER	DAYS AFTER OPERATION	LEVEL OF TRANSECTION	STIMULUS		LATENT TIME	TIME FOR ½ RELAXATION
			Coil	Duration		
Normal						
N-10		High	20	1.46	0.08	8.+
N-11		Undetermined	15	0 50	0.04	6.+
N-13		High	20	0 62	0.14	23 +
N-14		High	15	0 46	0 06	5 +
Deafferented						
D-10	24	High	15	1 46	0 16	0 5
D-11	22	High	10	0 86	0 12	0 36
D-12	20	High	12	0 84	0 10	0 78
D-15	13	Low	15	0 60	0 07	2 30
D-17	34	Low	10	0 70	0 04	7 +
D-30	26	High	10	0 80	0 16	0 76
D-21	35	High	20	0 86	0 06	0 94
		High	15	0 56	0 06	1 04
		Low	12	0 96	1 04	6 8
D-24	35	High	16	0 56	0 26	0 60
		High	10	0 66	0 28	2 02
		Low	10	0 88	0 62	15 92
D-27	36	High	30	4 04	0 24	0 66
		Low	30	1 68	0 16	6 7
		Low	16	2 06	0 56	13.+
D-28	30	High	17	0 78	0 20	0 78
		Low	16	1 66	0 11	14 42

Cats D-16 and D-19 of this series were used for the anemic decerebration and appear as cats D-7 and D-8 in table 1. The missing numbers in this series represent cats which developed ulcers of the deafferented right foot and were sacrificed or in which the decerebration was unsatisfactory, the animal either dying shortly after the transection or being in such shape that the reflexes could not be tested satisfactorily.

The latent time of the crossed extensor reflex in these experiments varied greatly but was with a few exceptions greater in deafferented than in nor-

mal muscles and greater after transection than after anemic decerebration (table 2).

In six operated cats the brain stem was cut across but once. In four of these the level of the section was high, i.e., it passed close to the rostral border of the superior colliculus and through the cerebral peduncles close to the mammillary bodies. In the two others the section was lower passing through the superior colliculus and through the cerebral peduncles close to the pons. In the four experiments with high transections, i.e., in cats D-10, D-11, D-12 and D-30 there was a rapid relaxation of the muscle following the crossed extensor reflex. The time required after the cessation of the stimulus for $\frac{3}{4}$ relaxation varied from 0.36 to 0.78 second. As shown in figure 1, contraction and relaxation were both rapid; and the reflex conforms to the type described by Sherrington as characteristic for deafferented muscle. In the reflex, recorded in this tracing, a brief period of after-discharge, lasting 0.18 second, followed the cessation of the stimulus. This after-discharge stopped abruptly and relaxation occurred so rapidly that only 0.36 second elapsed from the cessation of the stimulus before the relaxation was $\frac{3}{4}$ complete. The actual relaxation as measured from the end of the after-discharge occurred in half this time.

In the two experiments with transections made at a lower level (through the superior colliculus and through the cerebral peduncles a few millimeters in front of the pons) slower relaxations were obtained. In cat D-15, 2.3 seconds elapsed after cessation of the stimulus before the relaxation was $\frac{3}{4}$ complete and in cat D-17 over 7 seconds. It is of interest that, as shown in the list of levels on p. 57, the transection in D-15 was somewhat higher than in D-17.

It would appear that the level of the transection was an important factor in determining the rate of relaxation of the crossed extensor reflex. By the anemic method of decerebration all of the brain in front of the pons is thrown out of function (Pollock and Davis 1923). It is equivalent therefore, to a low transection and like the low transections in cats D-15 and D-17 yields tonic crossed extensor reflexes from deafferented muscles (fig. 2).

From these results it is evident that the character of the crossed extensor reflex in deafferented limbs depends on the level of the decerebration. After transections at the upper border of the mesencephalon a quick relaxation was obtained and after transection a short distance in front of the pons or after anemic decerebration the crossed extensor reflex was tonic and relaxed very slowly. Obviously the next step was to see if both types of crossed extensor reflex could be obtained from the same cat by successive high and low transections of the brain stem. This was accomplished with cats D-21, D-24, D-27, and D-28 with the results shown in table 2.

The second transection was done without ether and was not followed by shock. Immediately following the section decerebrate rigidity became more marked and the limbs stiffer than they had been before the mesencephalon was cut away. As soon as the animals could be again secured on the board good crossed extensor reflexes could be obtained although they were of a different type than they had been before.

In cat D-27, after a transection through the rostral border of the superior colliculus and just in front of the mammillary bodies, a crossed extensor reflex required 0.66 second for $\frac{3}{4}$ relaxation (table 2). This reflex was obtained at 11:20. At 11:22 the brain stem was again transected through the caudal border of the superior colliculus and the rostral border of the pons. At 11:30 a crossed extensor reflex was elicited by the same stimulus (secondary coil at 30) and required 6.7 seconds to reach $\frac{3}{4}$ relaxation. Three such tonic crossed extensor reflexes were obtained after the second transection. Similar results were obtained in cats D-21, D-24, and D-28. In each, at least three quick crossed extensor reflexes were obtained after high transection and three tonic responses with slow relaxation after low transection.

The marked difference in the crossed extensor reflex after high and low transection of the brain stem is illustrated by figures 3 and 4. Figure 3 illustrates a tracing from cat D-28 at 11:35 after section of the brain stem through the rostral border of the superior colliculus and the upper end of the cerebral peduncle. The relaxation was very rapid and the contraction curve makes a sharp angle with the curve of relaxation. At 12:05 a second section was made between the superior and inferior colliculi and through the rostral border of the pons. Five minutes later the crossed extensor reflex shown in figure 4 was obtained with slow contraction and much slower relaxation. Both responses were obtained from the same deafferented muscle and the marked difference between them is dependent on the level of decerebration. The slow relaxation is not due to deterioration of the muscle. At the end of the experiment the right popliteal nerve was stimulated with a current like that used in producing the reflex. The prompt relaxation which followed the contraction showed that the muscle was in good condition. The muscle was tested in a similar manner in a number of the other experiments with similar results.

Analysis of the contraction reproduced in figure 3 showed that the muscle continued to shorten 0.38 second after cessation of the stimulus due to after-discharge from the spinal cord and then promptly relaxed so that the relaxation was $\frac{3}{4}$ complete in 0.78 second after the end of the stimulus. The fact that the muscle was contracting against a heavy rubber band eliminates the possibility of any considerable momentum in the upstroke of the lever. Corrections were in every case made for the arc

of the lever. But it is not maintained that measurements of isotonic records can have more than relative values.

The crossed extensor reflex obtained after low transection and reproduced in figure 4 was tonic. The muscle shortened gradually and continued to shorten for 0.7 second after the end of the stimulus. It relaxed very slowly. Both contraction and relaxation were smooth and even. After the end of the stimulus 14.42 seconds elapsed before relaxation was $\frac{3}{4}$ complete. In the relaxation curve there appear two step-like drops, the second of these began 13 seconds after the end of the stimulus. Step-like drops of this sort occurred fairly frequently in the tracings of tonic crossed extensor reflexes in deafferented muscles but only slight indications of them were seen in the reflexes of normal muscles whether the decerebration was made by the anemic method or by transection, and even these slight traces were seen but rarely. The same peculiar relaxation in steps was seen in the crossed extensor reflex in deafferented muscle by Fulton and Liddell (1925). Each abrupt drop probably corresponds in time to the cessation of the after-discharge in a considerable number of motor neurones.

It seems probable that the prolongation of the crossed extensor reflex and the increased rigidity of the limbs, which followed the second transection, are directly related, in that both are due to a release of the tonic center in the hind brain from the control of a tone regulating center which is still at least partially intact after transection of the brain stem at the rostral border of the mesencephalon.

During the crossed extensor reflex the gastrocnemius and quadriceps contract simultaneously. It is conceivable that afferent impulses from the latter might reinforce and prolong the tonic contraction in the deafferented gastrocnemius. But this explanation of the prolonged contraction is ruled out by the fact that in one of our cats (D-21) the quadriceps was also deafferented by the section of all of the dorsal roots from the L IV to the S II inclusive.

It sometimes happens that the crossed extensor reflex does not appear until after the end of the stimulus or, as shown in figure 5, an abortive response may appear during stimulation, only to be suppressed at once by inhibition while the stimulus lasts, at the end of which it reappears in the form of an after-discharge. This atypical response was obtained from the deafferented right gastrocnemius of cat D-8, decerebrated by the anemic method. It is not, however, in any way characteristic of the reflexes obtained after this type of decerebration.

Another atypical reflex was occasionally obtained from deafferented muscle after high decerebration when strong stimuli were applied to the opposite popliteal nerve. One of these is illustrated in figure 6. Following the cessation of the stimulus, after-discharge maintained the response

at nearly its full height for a second or two and then ceased abruptly allowing the muscle to relax almost instantaneously.

The strength of the stimulus is an important factor in determining the duration of the after-discharge. By increasing the intensity of the stimulus the after-discharge may be increased 20-fold in the crossed extensor reflex of spinal animals (Sherrington, 1906). But in describing the rapid relaxation of the crossed extensor reflex which he obtained in deafferented muscles after decerebration, Sherrington (1909) says that prolongation of the contraction cannot be obtained by an increase in intensity of the stimulus. In our experiments, however, there does seem to be some relation between the strength and duration of the stimulus on the one hand and the duration of the contraction on the other.

In table 2 the relative strengths of the currents used are indicated by the position of the secondary coil and the duration of the stimuli is given in seconds. In cat D-24 after high decerebration with the coil at 16 the relaxation was $\frac{3}{4}$ complete in 0.6 second while with the coil at 10 the same degree of relaxation required 2.02 seconds (fig. 6). The same thing can be seen after low transection in the experiment on cat D-27 where with the secondary coil at 30 relaxation was $\frac{3}{4}$ complete after 6.7 seconds while with the coil at 16 the same degree of relaxation required more than 13 seconds.

But a study of the table will show that variations in the stimulus are not responsible for the marked difference in rate of relaxation after high and low transection. After high transections rapid relaxations were obtained with the coil at 10 in cats D-11 and D-30. In cat D-24 with the coil at 10 the relaxation was nearly eight times slower after low than after high transection. With the secondary coil at 30, cat D-27 gave a quick crossed extensor reflex after high transection and one ten times slower after low decerebration, although in the latter case the stimulus was of much shorter duration.

This prolonged response after a short stimulus makes it evident that while the duration of the stimulus may also influence the length of the after discharge it is not responsible for the differences seen after high and low transection.

THE CROSSED EXTENSOR REFLEX IN NORMAL CATS AFTER HIGH DECEREBRATION. In five normal cats the brain stem was transected just above the mesencephalon and used for the study of the crossed extensor reflex. The reflexes thus obtained were definitely tonic. The relaxations were much slower than those in deafferented muscle when the brain stem was transected at the same high level. It was more difficult, however, to secure the responses after high transection in normal than in deafferented muscle. In one of the five (N-12) no crossed extensor reflex could be obtained. In another (N-10) only one response, that recorded in the table, was ob-

tained, and in another (N-14) only two responses, after which repeated stimulation of the opposite popliteal nerve produced no response in the gastrocnemius. This might be interpreted as due to an early fatigue of the reflex arc in these cats. But after anemic decerebration, low transection, and, in the case of the deafferented cats high transection also, the five-minute interval between stimuli was sufficient for full recovery from fatigue and the responses could be repeated a great number of times. When a crossed extensor reflex was obtained from normal cats after high decerebration, it was definitely tonic as will be seen from the records given in table 2.

According to Girndt (1926) the crossed extensor reflex in thalamus animals has an essentially different character than in decerebrate preparations. The extensor muscle responds with a contraction which quickly subsides and gives place to a relaxation even below the original tonus level. This would harmonize with the work of Magnus (1924) and Rademaker (1926) which shows that there is in the extreme upper end of the mesencephalon a center for the regulation of tonus, which is capable of inhibiting decerebrate rigidity. In rabbits this center has been definitely located in the upper part of the mesencephalon but in the cat we believe that it extends upward into the tegmental portion of the hypothalamus (Hinsey and Ranson, 1928). It seems probable that after transection of the brain stem at the upper border of the mesencephalon this center is only partly destroyed and it may be responsible for the quick relaxation of the crossed extensor reflex in deafferented muscle and for the difficulty in obtaining the reflex in normal muscle.

SUMMARY

After anemic decerebration (ligation of the carotids and basilar arteries) the crossed extensor reflex is steady and tonic, the contraction continues for several seconds after the end of the stimulus and the relaxation is gradual. This holds true for the deafferented as well as for the normal gastrocnemius. Whether the presence of intact sensory fibers from the muscle increases the duration of the contraction cannot be determined from the records because the drum was not allowed to run for a sufficient length of time.

In animals decerebrated by the transection method it was found that the character of the crossed extensor reflex in deafferented muscle depended upon the height at which the brain stem was cut across. After transection at the upper border of the mesencephalon the reflex lost all evidence of tonicity. Relaxation followed abruptly the cessation of the stimulus. But if the transection were made at or near the upper border of the pons the deafferented gastrocnemius responded to stimulation of the opposite popliteal nerve by a prolonged tonic contraction similar to that

seen after anemic decerebration. Low transection and anemic decerebration give identical results as might be expected from the fact that ligation of the carotid and basilar arteries renders all of the brain in front of the pons functionless because of anemia.

It is possible to obtain from one and the same deafferented muscle an atonic crossed extensor reflex after high decerebration and a few minutes later a tonic crossed extensor reflex after a second transection of the brain stem at a lower level.

After high transection in normal cats the crossed extensor reflex is tonic but in our experience has been more difficult to obtain than after low decerebration.

In the upper end of the mesencephalon there is a center for the regulation of tonus which Rademaker has identified with the red nucleus. In cats it seems probable that this center extends upward into the hypothalamus. Through the brachium conjunctivum the cerebellum makes important connections with the red nucleus. In low decerebrations all of these tegmental structures are cut away and their inhibitory influence on the tonic center in the hind brain is lost. When this source of inhibition has been cut away the deafferented gastrocnemius yields a tonic crossed extensor reflex. When the tegmental portion of the mesencephalon with its cerebellar connections is intact the deafferented gastrocnemius yields an atonic crossed extensor reflex due to the suppression of the after-discharge. Whether the cerebellum plays an essential part in this inhibition has not been determined.

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EFFECT OF CORPUS LUTEUM AND OVARIAN EXTRACTS ON THE OESTRUS OF THE GUINEA PIG

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The earlier work on the endocrinology of the ovaries beginning with the classical publications of Fraenkel (1) and Loeb (2) has emphasized the important endocrine function played by the corpus luteum and this work has profoundly influenced subsequent investigations on ovarian hormones for a long time. With the discovery by Stockard and Papanicolaou of a new method of investigating the sexual cycle of rodents by means of vaginal smears and the extensive elaboration of this method by Allen and Doisy (3) in this country and Zondek (4), Loewe (5) and Laqueur (6) and others in Europe, the pendulum has swung in the opposite direction so that the popular and prevalent tendency is at present to stress the endocrine importance of ovarian follicles. The present authors have been interested for many years in the chemical, physiological and pharmacological studies of ovarian hormones in general and of corpus luteum in particular, and in the present investigation have undertaken on an elaborate scale the intensive study of corpus luteum in this respect employing the newer methods of following the oestrous cycle of guinea pigs by means of repeated vaginal smears.

METHOD. The present investigation has been carried on for a period of about four years. Two series of experiments dealing with the effect of injections of corpus luteum extracts were carried out. The chemical part of both series was performed by Mr. Arthur Stickel, the first series of physiological experiments was conducted by Dr. D. L. Seckinger and was carried out on a total of fifteen guinea pigs. In the second series the biological work was repeated and extended under the direction of Doctor Macht and the first set of experiments was repeated and checked up. In addition, numerous special controls with injections of other extracts were performed. It may be stated at once that the results of the second and more extensive series of experiments in which at least twenty-five animals were utilized not only corroborated the original findings but strengthened and extended them.

Inasmuch as the biological part of the research depended chiefly on the

method of vaginal smears great care was taken in selecting the animals before beginning studies of the oestrous cycle. In both series of experiments young virgin and perfectly healthy guinea pigs were selected which had been in oestrus some six to ten times before coming into the laboratory. All diseased animals were rejected because, as will be shown later, infections and other abnormalities seriously affected the sexual cycle. The animals were kept under uniform conditions in clean cages on a liberal diet of hay, grain and greens, and in addition the animals were occasionally given small rations of whole milk. After the oestrous cycle for each animal was established by the smear method over a period of many weeks, as described in the following paragraph, injections of the extracts were begun. Such injections it was found best to begin soon after the animal passed through an oestrus. In other animals, however, injections were begun several days later during the dioestrus. Most of the injections were given under the skin after preliminary sterilization to prevent infection. Occasionally intraperitoneal injections were given.

The microscopic study of vaginal smears as an index to the sexual cycle of animals dates from the studies of Stockard and Papanicolaou on the guinea pig, published in 1917, and in the present investigation the authors were guided almost entirely by the original methods of these authors. Four different stages are noted in the study of vaginal contents during the oestrus of guinea pigs, these stages being characterized by typical microscopic findings which need not be described in this place. For practical purposes in the present study the height of the oestrus and its duration could be easily determined by its number of desquamated epithelial cells and leucocytes.

The experience of the present writers while agreeing completely with the microscopic findings of Stockard and Papanicolaou has shown, however, that the duration of the sexual cycle is quite variable in different individual guinea pigs (7). In this the findings of the present authors are more in agreement with the recent extensive investigation of H. E. Voss (8) who also studied the oestrous cycle of guinea pigs by means of vaginal smears. The authors have found that the duration of the dioestrus in some guinea pigs was much shorter than in others, varying from eight to fifteen days in length. On the other hand, however, it must be emphasized that for any one individual guinea pig the oestrous cycle is pretty regular and constant under normal conditions and that it changes only if the animal becomes diseased or is treated with injections of certain extracts. The guinea pig was selected in the present investigation in preference to mice and rats used in much of the recent work on ovarian hormones because the sexual cycle of the smaller animals is peculiar in that the lutein phase is developed only in a very rudimentary fashion and furthermore the oestrous cycle of these rodents is of very short duration; the guinea pig on the other

hand exhibits a dioestrus of a much longer duration so that the effect of injections in these animals can be gauged much more readily.

PREPARATION OF LUTEIN EXTRACTS. The solutions employed in the present investigation were prepared by several methods. The products obtained by the different methods were all tested and it was found that two of them gave the most active preparations. Such lutein solutions giving the best results were the ones employed for the study of its effects on the oestrous cycle of the guinea pig and were prepared as follows:

1. The first solution employed was prepared in the following manner: corpus luteum powder was exhaustively extracted with petroleum ether and the residue further extracted with ether. The powder was then macerated 24 hours with five times its weight of a chloroform saturated aqueous solution of 0.1 per cent sodium chloride and 0.1 per cent sodium bicarbonate. The liquid was then centrifugalized and decanted. This was repeated four times. To the combined extracts sufficient 94 per cent alcohol was added to make the alcoholic strength 60 per cent. Protein was removed by successive precipitation with normal lead acetate solution and Goulard's solution. Excess lead was removed by adding dry sodium bicarbonate and filtering. The clear solution then was neutralized with hydrochloric acid, using litmus, and evaporated almost to dryness in an air dryer at 100° to 110°F. The residue was then taken up in a sufficient amount of 0.5 per cent chloretone solution to represent 0.2 gram of original dried gland per cubic centimeter. This solution was then adjusted to neutrality, using litmus, and then clarified by filtration. This solution was water soluble.

2. The powdered glands were treated with petroleum ether and ether as in 1, and then agitated with 99 per cent ethyl acetate for 24 hours. About 6 cc. of ethyl acetate were used for each gram of gland. Three or four such extractions were made and the combined extracts were evaporated to dryness in a glass capsule at 100° to 110°F. in an air dryer. The residue obtained amounted to 0.4 to 0.6 gram for every 100 grams of powdered gland used. To this was added a sufficient amount of 0.5 per cent chloretone solution to correspond to a concentration of 1 gram of dry gland per cubic centimeter. A slight excess of dry sodium bicarbonate was added and the residue dissolved *by rubbing with a glass rod.* Hydrochloric acid was added until the solution became neutral to litmus, and clarified by filtration. Each cubic centimeter contained 4 to 6 mgm. of extractive. At first only hand-dissected corpora lutea were used, but later it was found that the commercial preparation was satisfactory.

The ethyl acetate extractive contains the active principle in the purest form yet obtained and was employed in most of the experiments. The extract thus obtained was active even after boiling the ethyl acetate in a reflux condenser on a water bath for two periods of six hours each. It is

water soluble, the aqueous solution giving a very slight acid reaction of litmus. On chemical analysis this extract was found to be free from phosphorus and to contain less than 2 per cent nitrogen.

Various other methods of extraction were also tried which need not be discussed in this place as they did not yield as good a product as those above. For controls extracts of various other glands and tissues were prepared in exactly the same way as described in the second method for corpora lutea. In this way the authors have prepared water-soluble extracts of powdered beef muscle, of powdered thyroid gland, of powdered

TABLE 1

Effect of corpus luteum extracts

GUINEA PIG NUMBER	FIRST SERIES (PERFORMED 1924-1925)			GUINEA PIG NUMBER	SECOND SERIES (PERFORMED 1926-1928)		
	Preparation given number	Normal interoestrus (days)	Days of inter-oestrus		Preparation given number	Normal interoestrus (days)	Days of inter-oestrus
A	1	13	29	A	11	10	30
B	1	12	25	B	11	9	22
B	1	12	22	C	11	9	30
C	11	12	64	D	11	15	33
D	11	14	48	E	11	9	20
E	11	13	59	F	11	9	22
B	11	12	42	G	11	9	22
F	11	13	30	H	11	10	23
F	11	13	30	I	11	15	30
G	11	13	46	J	11	9	22
H	11	14	14	K	11	15	41
A	11	13	39	L	11	11	33
I	11	12	47	M	11	12	36
D	11	14	29				
Average.....		13	37.4	Average.....		10.9	28

suprarenal cortex, of powdered pituitary gland (anterior lobe) and of powdered cryptorchids.

EFFECT OF CORPUS LUTEUM EXTRACTS. The effect of injections of corpus luteum extracts in the guinea pigs is exhibited in table 1. The first series of experiments was performed in the years 1924–26 and is given in table 1. The second series of experiments was performed on an entirely new series of guinea pigs in the years 1926–28 and the results obtained are also indicated in the same table. The preparation of corpus luteum used in most of the experiments was the one prepared by the second method described and is marked as no. II. In the first experiments preparation I was also used which was made by the use of lead acetate solution as described above. Both of these preparations were water-soluble. It will be noted in the

first series that while the average oestrous interval was normally thirteen days, after injection of corpus luteum extract an inhibition of the oestrus was noted with the exception of experiment I. The average duration of the interoestrous for the guinea pigs which received injections in this series was 37.4 and indicates a prolongation of the interoestrus by 187 per cent. It was found as indicated in experiment fourteen that heating the corpus luteum extract at 100°C. did not affect its potency. Most of the injections were begun a day or two after an oestrous period in the guinea pig and the injections were given in some cases daily and in other cases on alternate days, both methods of injection giving very much the same results. The second series of experiments was undertaken in order to check up on the first series and it will be noted that the results obtained agreed completely with those obtained in the first series. The average duration of normal interoestrus in this series of pigs was 10.9 days while the average duration of the interoestrus after injections of the corpus luteum extracts was twenty-eight days, thus showing a prolongation of the interoestrual period of 157 per cent. In the second series of experiments it was found that the oestrous cycle of the guinea pigs was not as uniform as in the first series although the cycle for each individual animal was quite regular. This agrees with recent findings of Voss. The corpus luteum extracts which were injected into the above animals were obtained from the fresh corpora lutea of the sow. A few experiments were made with an extract prepared in a similar way as the above from fresh glands of the cow. These were found to be of the same potency as the pig extract. The average *duration* of oestrus in normal guinea pigs is three to four days. The average duration of oestrus after administration of corpus luteum is one to two days.

The inhibition of the oestrous cycle obtained with the corpus luteum extract was very marked when the results were compared with those obtained with control solutions. Control experiments were made with extracts of various tissues and glands. In this way extracts of beef muscle, thyroid glands, anterior lobe of the pituitary and cryptorchid glands were studied and were found to give absolutely negative results. None of these produced inhibition and in fact some of them seem to shorten the interoestrous interval. This is, of course, of interest in this connection.

Effect of other ovarian extracts. While the authors were primarily interested in a physiological study of the corpus luteum, in order to obtain a more comprehensive view of its properties experiments with other ovarian extracts were performed. Thus experiments were made with extracts of follicular fluid. Some of these were prepared according to the method of Allen and Doisy and were of an oily character. Other follicular extracts were prepared by a method similar to the one employed for corpus luteum and yielded a water-soluble preparation. In the same way a water-soluble

preparation of the whole ovary was prepared, and again in a similar way a water soluble preparation of ovarian residue was obtained. The effect of injections of such extracts is exhibited in table 2. It will be noted that the injections of follicular extract shortened the dioestrus and brought on the oestrous picture, as indicated by the examination of the smears, to a very marked degree. There was no difficulty whatever in inciting a condition of oestrus as indicated by vaginal smears with injections of follicular extracts. The effect of whole ovary tended to prolong the dioestrus as a rule but in some cases, probably owing to the presence of an excessive amount of the follicular hormone, the interoestrous interval was shortened. Injections of ovarian residue extract did not seem to produce any effect on the oestrous cycle of the guinea pig in the few experiments in which this preparation was administered.

TABLE 2

Effect of other ovarian extracts

EXPERIMENT NUMBER	NORMAL DIOESTRUS	DIOESTRUS AFTER FOLLICULAR EXTRACT	DIOESTRUS AFTER WHOLE OVARY EXTRACT	DIOESTRUS AFTER OVARIAN RESIDUE	DIOESTRUS AFTER CORPUS LUTEUM EXTRACT
		days	*days*	*days*	*days*
1	16	2		15	30
2	15	3			33
3	12			16	30
4	9		20		30
5	9		13		22
6	10		8		30
7	15	2		15	33
8	15	Continuous			30
9	15	2		15	30

EXPERIMENTS WITH THE VAS DEFERENS. Macht and Matsumoto (9), in connection with a study of the effects of various endocrine products on the movements of genito-urinary organs, discovered that the muscular contractions of the vas deferens are particularly sensitive to very small doses of corpus luteum extracts which caused them to contract to a greater degree than any other endocrine preparation with the exception of epinephrin. It has been a routine practice in this laboratory to examine the physiological activity of corpus luteum products by determining the stimulating action on the isolated vas deferens of the rat and expressing it in terms of a contraction produced by a standard dose of epinephrin. According to a large amount of clinical information obtained by the authors from numerous gynecologists the preparations of corpus luteum which in their opinion gave the best therapeutic results were found to be most active in their action on the vas deferens. In this connection it was deemed worth

while to compare the physiological activity of corpus luteum extracts as indicated by studies of vaginal smears from guinea pigs with the relative figures obtained with the same preparation on the isolated vas deferens of the rat. The figures thus far obtained seem to indicate that the effect of corpus luteum extracts on smooth muscle of the vas deferens parallels their inhibitory effect on the oestrus of the guinea pig as may be illustrated by table 3.

It will be noted that where no inhibition of the oestrus occurred no contraction of the vas deferens was noted and that where inhibition of the oestrus did occur the degree of inhibition ran parallel to the activity of the preparation for the vas deferens. The standard method employed by the authors in performing the vas deferens test was to compare the effect of 1 cc. of the extract in 50 cc. of Locke's solution with the contraction produced by 0.1 mgm. of epinephrin in 50 cc. of Locke's solution for the same vas. The results of the present investigation concerning the action of corpus luteum based on the study of vaginal smears and reactions of the same

TABLE 3

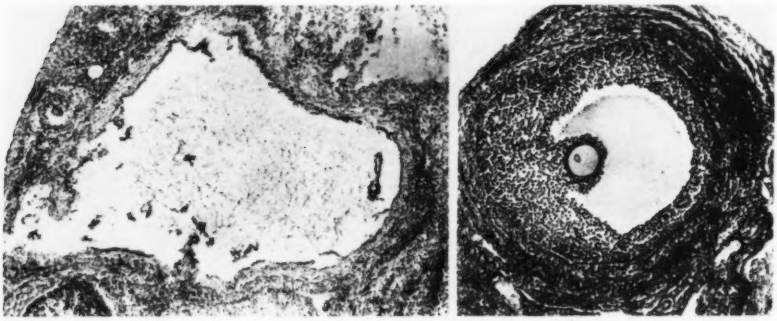
Comparison of vas deferens and vaginal smear observations

GUINEA PIG NUMBER	AVERAGE NORMAL INTEROESTRUS	AVERAGE INTEROESTRUS AFTER INJECTION OF LUTEIN	DEGREE OF OESTRUS INHIBITION	CONTRACTION OF VAS DEFERENS IN TERMS OF STANDARD EPINEPHRIN DOSE
			per cent	*per cent*
3	8	18	250	75
17	6	29	400	150
15	6	18	300	100
2	7	7	0	0

on the muscle of the vas deferens indicate that corpus luteum extract possesses physiological properties different from follicular extracts and the investigation, therefore, speaks in favor of at least two hormones to be derived from the ovaries.

ANATOMICAL FINDINGS. The authors have endeavored to some extent to follow the anatomical change in the ovaries of guinea pigs in connection with the studies of vaginal smears. For this purpose a number of guinea pigs were sacrificed and their ovaries examined. Thus in one guinea pig injections of 1.5 cc. of corpus luteum extract were begun on the day after oestrus. Injections were continued every other day and on the thirteenth day when ovulation should normally have occurred the guinea pig was killed. A macroscopic examination of the ovaries showed no corpora lutea and only minute follicles which were almost invisible to the naked eye. As a control another guinea pig which was not receiving any treatment was also killed on the thirteenth day after oestrus. The ovaries of this guinea pig showed a mature Graafian follicle. This experiment was repeated

again with similar findings so that there seemed to be an actual inhibitory effect demonstrable anatomically after injections of corpus luteum extracts. These studies were carried a little further. A number of guinea pigs were treated with injections of corpus luteum extract and an inhibition of the oestrus was noted. Two such guinea pigs were killed and microscopical sections of the ovaries were prepared. As controls two other guinea pigs were killed at a time corresponding to their oestrous cycle and histological sections of their ovaries were also prepared. A microscopic examination of the sections revealed a decided difference between the control and treated guinea pigs. The picture obtained is illustrated in figure 1. According to the opinion of an eminent gynecological histologist who examined the sections, the difference between the two ovaries was as follows. "There was a decided decrease in the number of maturing follicles in the treated



a b

Fig. 1 a. Enlargement of treated ovary. Magnification 120. b. Enlargement of control section.

guinea pig as compared with the control sections examined and in addition there was a tendency to a development of atretic follicles in the treated guinea pig's ovaries." The significance of these findings will be discussed further on.

EXPERIMENTS WITH PLACENTA EXTRACTS. In the opinion of certain writers the placenta is to be regarded as possessing to some extent functions not unlike those of the corpus luteum, that is, one of its purposes is to inhibit the sexual cycle of higher animals. Thus Bandler writes, "Since its relation to the corpus luteum is of a stimulative nature and the corpus luteum and the placenta have a somewhat similar function" (10). Beard suggests that the placenta may have a function of inhibiting ovulation (11) and the same opinion is expressed by Carlson (12). More recently Kovacs has published experimental data indicating that injection of placental pulp

will prevent pregnancy in white rats (13). The authors have performed a few experiments with extracts of the placenta of the sow prepared by the same method as the extracts of the other glands studied. The results obtained are exhibited in table 4 It will be noted that in five guinea pigs out of six there was some inhibition of the oestrus after injection of placental extracts. Only in one of the animals, no. 4, was no change noticed.

TABLE 4

Effects of placental extracts

EXPERIMENT NUMBER	NORMAL INTEROESTRUS (DAYS)	DAYS OF INTEROESTRUS
1	14	39
2	13	40
3	12	27
4	14	14
5	9	15
6	15	25

Discussion. Irrespective of the various theories held by different authors in regard to the number of hormones present in the ovary, whether one or two or three (14), the results obtained in the present investigation indicate unequivocally a physiological activity on the part of corpus luteum extracts in relation to the oestrous cycle of the guinea pig. The present authors have found that injections of such extracts prolong the interoestrous period, and also shorten the duration of the oestrus when it occurs. This was learned from microscopical examination of vaginal smears and also through anatomical studies. These results seem to be in perfect accord with the original theories of Fraenkel and Loeb who held that one of the functions of the corpus luteum was to inhibit ovulation. These experiments also confirm Loeb's later experiments on guinea pigs along the same lines in which he showed that by extirpating the corpora lutea ovulation occurred sooner than normally (15). According to certain veterinarians it has been found that atretic follicles seem to inhibit conception in mares and produce sterility. When these and the corpus luteum are removed surgically such animals are said to conceive and become pregnant (16). Very recently Corner undertook to repeat and extend the experiments performed years ago by Fraenkel and Ancel and Bouin (17) who had demonstrated the occurrence of anatomical changes in the uterus correlated with the development of the corpora lutea on the one hand and the excision of the corpora lutea on the other hand. The conclusions arrived at by Corner were that his experiments supported the findings of Bouin and Ancel completely and also confirmed Fraenkel's views in regard to the corpora lutea so that Corner concludes that these results "point

strongly to the extension of a special action of the corpus luteum upon the endometrium bringing about progestational proliferation" (18). Pearl and Surface reported work supporting an inhibitory effect of corpus luteum on ovulation in the fowl with corpus luteum extracts (19). Other writers who have held this view as to the function of the yellow bodies are C. T. Zuntz (20), H. Ruge (21), Krobansky (22) and T. P. Sandes (23). Beard in 1897 on theoretical grounds, based upon the data of comparative embryology and phylogeny, attributed to the corpus luteum the function of regulating the onset of labor and of preventing ovulation (24). Papanicolaou reports delayed oestrus in the guinea pig after injections of corpus luteum extract (25). Parkes and Bellerby (26) have recently succeeded inhibiting oestrus in a non-castrated mouse by injecting an acetone soluble extract of cow's corpora lutea. Payne, Van Peenan and Cartland (27) produced inhibition of oestrus in rats by injections of corpus luteum extracts and even Loewe and Lange (28) found a slight inhibitory action in mice after injection of corpus luteum extracts. Finally Professor Gley, in one of his latest publications, writes that by injecting very potent corpus luteum extracts in rats he has been able to inhibit the oestrus indefinitely so long as the injections have been continued (29).

To the present authors it would seem that the evidence for endocrine function of the corpus luteum is certainly as positive as the evidence produced by recent writers for the other hormone of the female gonads residing in the follicular fluid. The present writers by injections of follicular extracts have been able very easily to confirm the findings of Allen and Doisy, Frank and numerous other writers in regard to stimulation or hastening of oestrus not only in rats but in guinea pigs. The very universality of the hormones of Allen and Doisy, however, renders its demonstration by means of vaginal smears less specific than the demonstration of inhibition of the cestrus by corpus luteum extracts. A picture similar to that obtained after injections of liquor folliculi has been shown to follow injections of extracts of all kinds of other organs and tissues. Loewe (30) and and Frank (31) found this sex hormone in the blood of women and other animals; Laqueur (32) finds it in the liver; Doisy, Ralls, Allen and Johnston (33) find it in the pancreas, and Fellner (34) and others find it in the testis. Loewe (35) found it in the urine of women and the hormone has been found also to be present in certain extracts of plants. An *inhibition* in the oestrous cycle of guinea pigs, however, could not be produced by the present authors when injections of any extracts except those of the corpus luteum and possibly those of the placenta. The effect of extracts of the whole ovary in inhibiting oestrus in some of the experiments can be explained by its corpus luteum content.

In connection with the present investigation the authors made an interesting observation to which attention of other workers should be directed.

In following the sexual cycle of guinea pigs by means of vaginal smears, it is absolutely necessary to employ perfectly healthy normal animals. It was noted that when animals developed even a slight infection of a pyogenic nature, for instance from the use of a non-sterilized needle, the microscopic picture given by the animals was completely changed. The oestrus became very irregular and interoestrous intervals were much shorter and also irregular.

A physiological activity of corpus luteum extracts is shown not only by the effect on the oestrous cycle but by certain other biological changes induced by the employment of such extracts. Macht and Seago (36) have studied the psychological effects of ovarian extracts on rats by means of the circular maze and found that injections of corpus luteum extracts produced a stimulation of the neuromuscular apparatus. Again it is well known that there are definitely established menstrual psychoses which seem to be connected with the sexual cycle of the ovaries. An interesting contribution on this subject has been published recently by Prengowski (37) in which the author described ten cases of menstrual psychosis. Seven of these, having a diminution of the periods, exhibited mental disturbances of *hypomanic* to delirious type and were cured by injection of ovarian extracts which tended to stimulate the menstrual function. The other three cases, having an augmentation of the periods and also suffering from mental disturbances, were successfully treated by an extract of the ovaries which tended to inhibit the function. Another interesting physiological property of corpus luteum extracts in vitro was studied by Seckinger (38) who found that the effect of such extracts on isolated surviving smooth muscle of the fallopian tubes produced different types or patterns of contractions depending on the pregnant or non-pregnant state of the organ, by treatment with corpus luteum extracts.

SUMMARY

1. Specially prepared water-soluble extracts of corpus luteum were studied in respect to their effect on the oestrous cycle of guinea pigs by the vaginal smear method.

2. Injections of such extracts produced inhibition of the oestrus and such inhibition of the oestrus was accompanied by characteristic histological findings.

3. Inhibition of the oestrus could not be produced by injections of other glandular extracts with the exception of the placenta which also tended to inhibit the oestrous cycle.

4. Attention is drawn to the disturbing effect of infections on the oestrus of the guinea pig which are liable to lead to erroneous conclusions on study of vaginal smears.

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ACTION OF FORMALIN AND HISTAMINE ON TENSION AND POTENTIAL CURVES OF A STRIATED MUSCLE, THE RETRACTOR PENIS OF THE TURTLE

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Opposing actions of certain aldehydes and histamine, upon smooth muscle of the gut, the aldehydes relaxing histamine contracture, have been reported by Kendall (1927). A corresponding difference has been shown by the present authors (Bishop and Kendall, 1928) in the response of smooth muscle stimulated electrically after treatment with these chemicals in doses below threshold for a direct effect on muscle length. Verzar (1914) found that skeletal muscles treated with aldehyde gave a typical veratrin-like contraction, a result which seems to contrast with the effects of formalin on smooth muscle. The present paper deals with a comparison of the effects of formaldehyde and histamine upon a striated muscle, the retractor penis of the turtle, and with the relation between the action potential and the contraction.

This muscle is a long narrow one running (bilaterally) from the anterior surface of the cloaca to the spine, through various muscle loops. It lies free in a sheath, however, and is easily removed without injury. Its properties have been studied by Hofmann (1913). It is parallel fibered, the impulse being conducted in at least some fibers throughout its length, as may be shown by splitting the two ends, stimulating one branch, and recording the contraction of the corresponding branch at the other end. When first removed it relaxes under slight tension to at least three times its normal length in the body. Its action is slow, its conduction rate about 2.5 m.p.s. at 25°C. as measured by its action potential, in the extended muscle. Hofmann cites a figure of 0.41 m.p.s. at 22°, but does not state extension. Hofmann recorded both action potentials and contractions. The action potentials are discrete under repeated stimuli, but the contractions are fused even at relatively slow rates of stimulation to a smooth tetanus. The motor point is near the middle.

Examined histologically the muscle is distinctly and finely cross striated, especially when fixed in the contracted state. The fibers are loosely embedded in a very elastic connective tissue, vary considerably in size, and

end spindle-shaped in a fascia at the penis attachment. Aside from diameter the fibers appear to be all alike. It is very irritable to induction shocks, and is extremely rugged, physiologically speaking, showing but slight deterioration after long stimulation outside the body. Its mono-

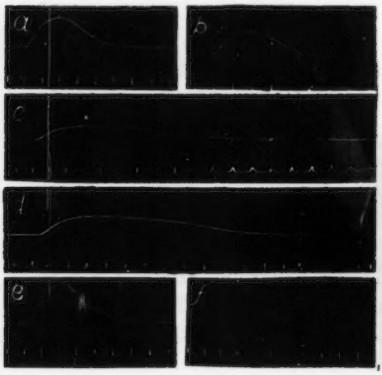

Fig. 1

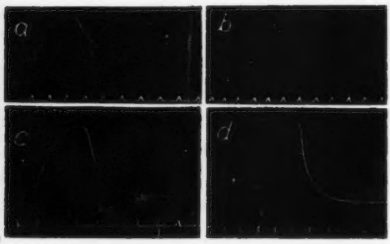

Fig. 2

Fig. 1. Turtle retractor penis washed with 1:100 solution of 30 per cent formalin (a strong solution). Hung on a spring muscle lever, stimulated by condenser, discharges through non-polarizable electrodes with yarn wicks. A segment of the penis to which the muscle was attached was clamped, about one-half the muscle being used (3–4 cm. extended). The lower end of the muscle was tied, the thread forming a loop that could be readily detached from the lever for washing. Amplitudes may not be quite comparable when the muscle had been removed during a series (i.e., between *a-d* and *e-f*). Threshold at start, 0.06 mf. at 1.5 v. *a*, normal, 0.4 mf. at 1.5 v. *b*, once washed with 1:100 formalin, 0.4 mf. *c*, twice washed, 0.3 mf. (threshold 0.10 mf.). *d*, thrice washed, 0.4 mf. (threshold 0.06 mf.) 10 minutes' duration of experiment between *a* and *d*. To Ringer's solution over night in ice box, then threshold 0.14 mf. at 1.5 v. *e*, 0.6 mf. at 3 v. *f*, 0.8 mf. at 3 v. Complete recovery with the exception of the higher stimulus required. Room temperature about 25°C., time in 0.2 second. One centimeter in amplitude measures about 10 grams tension. The free period of the lever was 15 per second. One-half natural size.

Fig. 2. Turtle retractor penis, washed with histamine 2:100,000. Arrangement, time, and method of stimulation as in figure 1. Muscle from opposite side of same turtle. *a*, normal, stimulus 0.2 mf. at 1.5 v. threshold 0.04 mf. *b*, 3 minutes after washing with histamine. Stimulus 0.7 mf., threshold 0.09 mf. *c*, 15 minutes after *a*, washed again with histamine, stimulus 0.7 mf., threshold 0.08 mf. *d*, 20 minutes after *a*, washed again with histamine, stimulus 0.7 mf., threshold 0.06 mf., all at 1.5 v. One-half natural size.

phasic action potential recorded near the stimulated point is like that of other skeletal muscle (Bishop and Gilson, 1927), and consists of an initial peak followed by a prolonged fall, approximately coterminal with tension development in the muscle. This muscle is presumably *not* homologous with the retractor penis of the dog, which has quite different characteristics.

The muscle is thus a useful one for physiological study due to its form, its slow contractile process, and its extreme endurance. The only serious drawback connected with its use arises from its association with the sex of the animal. In the long run our turtles have been about half males,

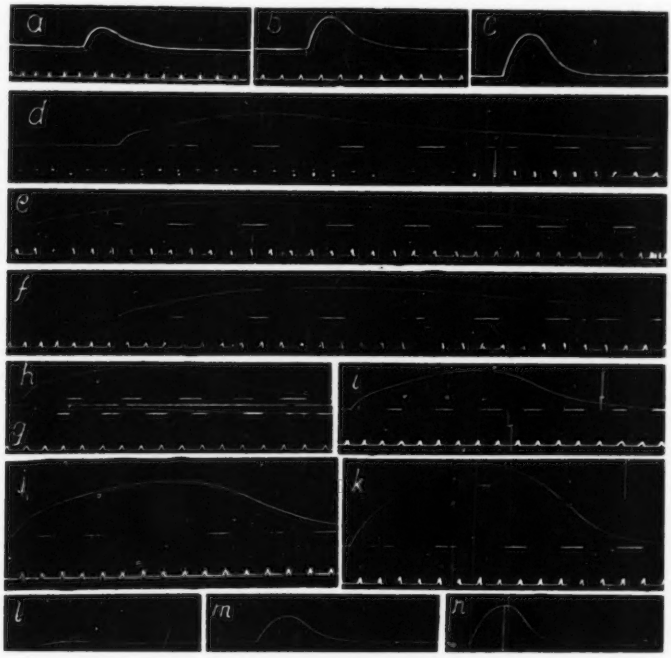

Fig. 3. Turtle retractor penis, kept wrapped in moist filter paper in ice box over night before experiment. Washed with 1:500 30 per cent formalin. Arrangement, time, etc., as in figure 1. Records reduced $\frac{1}{2}$ in reproduction. *a*, stimulus 0.05 mf. at 22 v. *b*, 0.2 mf. at 22 v. *c*, 0.1 mf. at 45 v., threshold 0.02 mf. at 22 v. Normal. *d*, after formalin, 0.1 mf. at 22 v. *e*, 0.08 mf. at 6 v. *f*, 0.10 mf. at 6 v., threshold 0.02 mf. at 22 v. To Ringer's solution, washed 20 minutes, then, *g*, stimulus 0.03 mf. at 6 v. *h*, 0.1 mf. at 6 v. *i*, 0.1 mf. at 22 v. To Ringer's solution, washed 45 minutes, then *j*, 0.06 mf. at 6 v. *k*, 0.4 mf. at 22 v. To Ringer's solution 200 minutes, then *l*, 0.03 mf. at 6 v. *m*, 0.05 mf. at 6 v. *n*, 0.4 mf. at 6 v. drum speed as above. Recovery apparently complete.

but in one sequence of nine, the law of probabilities failed us with respect to the first eight. The presence of the muscle in question can, however, be predicated by the employment of a suitable proctoscope. The criteria of sex proposed by Hofmann do not appear to obtain for our material.

Tension curves. This muscle when washed with dilute solutions of

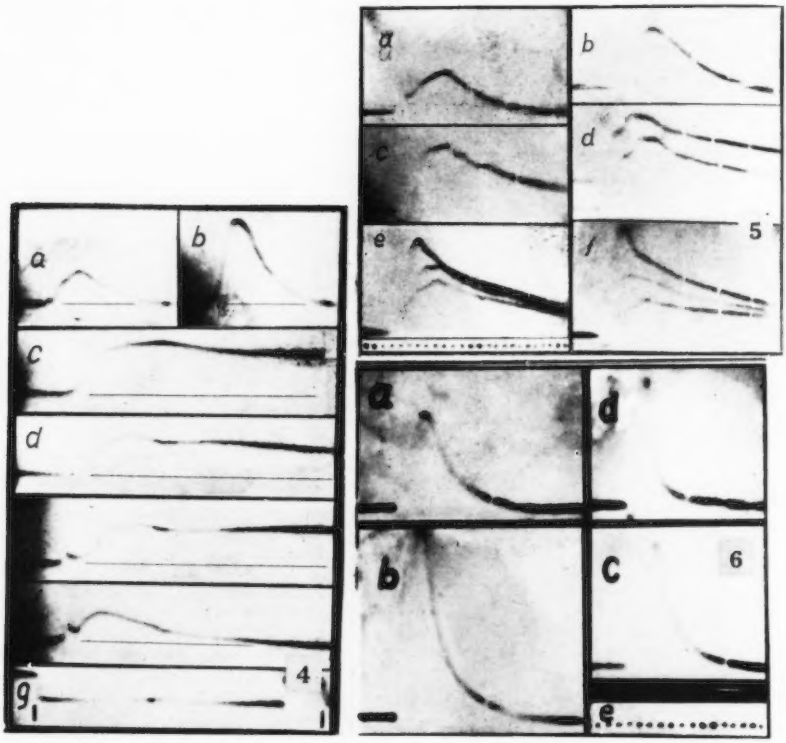

Fig. 4. Cathode ray oscillograph records of turtle retractor penis potentials under formalin. Single responses to induction shocks 2 mm. from site of stimulation, isometric. 1:500 30 per cent solution formaldehyde in Ringer's. *a*, weak stimulus, *b*, strong, untreated, *c*, *d*, *e*, successive responses at increasing strengths of stimulus after 1½ hours' soaking in formalin-Ringer's solution, *f*, after 1 hour washing in Ringer's maximal stimulation; *g*, time between marks, 0.2 second. Time approximately linear. The muscle failed to contract detectably after treatment, but, after recovery at least, the electrical impulse was conducted with decreased amplitude.

The shocks for these responses were applied *across* the end of the muscle, the proximal lead being placed between and a short distance from the stimulating electrodes; this arrangement (Bishop and Gilson, 1927) minimizes the shock record, which distorts the muscle record in these figures not later than the first third of the rising phase. A Porter coil was used, without the core. With low resistance through the muscle, these shocks have an effective duration of several ten-thousandths of a second, and the polarization resulting in the muscle lasts several times that long. Unless very small and well compensated (*a*, *b*, *c*, above) the shock record is too fast to photograph by single deflections of the oscillograph.

Fig. 5. Retractor penis under formalin, 1/1000, potentials as in figure 4. *a*, submaximal stimulus, immediately after washing with formalin, *b*, 11 minutes later after

formaldehyde promptly undergoes a change in its tension curve very like that undergone by a veratrinized skeletal or heart muscle (figs. 1, 3). Stimulated by condenser charges, its response alters from a simple typical skeletal muscle response to a double-humped contraction, whose second phase may be greatly prolonged, and considerably higher in amplitude than the first. After long washing in Ringer's solution (several hours) this change is completely reversible, and the muscle has undergone no obvious deterioration.

If, instead of washing in Ringer's solution, histamine is washed over the formalin treated muscle, the veratrin-like action of formaldehyde is neutralized slowly, but the combination seems to cause more deterioration than does either chemical alone.

When the muscle is treated with histamine first, the opposite effect is seen in slight degree, in that the (maximal) contraction is higher, and if anything, shorter, though this change is not pronounced (fig. 2, *d*). Formaldehyde solution reverses the action of histamine, with the typical veratrin-like response.

Action potentials. This muscle is particularly suitable for recording action potentials. It is long and thin, and conducts well. It is very sensitive to short induction shocks, and so slow in response that the shock distortion is over before the muscle has more than begun to respond. Stimulated near the cloacal attachment, far from the motor point, with a lead at or close to the stimulus (1 to 3 mm.) action potentials from muscles freshly killed by pinching a centimeter or more away appear as in figure 4, *a b*. After the action of formaldehyde the latter part of the potential is as greatly prolonged as is the contraction, but the initial peak is not materially changed at first. The alteration is reversible by washing (figs. 4, 5).

Histamine applied to the previously untreated muscle results in the opposite effect on the action potential (fig. 6). The later portion of the potential wave is progressively reduced, until it is scarcely noticeable, but can be demonstrated to be present by allowing the wave to be recorded at high sensitivity of the apparatus, permitting the first peak to pass far off the record. Washing with Ringer's solution restores the muscle only partially within a reasonable time, but formaldehyde reverses the effect.

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further washing, *c*, 9 minutes later, *d*, after 30 minutes in Ringer's; *e*, print through three records superimposed, increasing stimuli, potentials of two higher are shunted. The form changes with strength of stimulus, the stronger shock increasing the first spike of the potential more than it does the following portion. *f*, same as *e* after one half hour further washing. Time (on *e*) in 0.01 second.

Fig. 6. Retractor penis as in figure 4. Histamine acid phosphate 1/250,000. *a*, normal, submaximal shock, *b*, 7 minutes after washing with histamine, maximal stimulus, reduced to less than ½ amplitude by shunts; *c*, 2 minutes later, submaximal, unshunted; *d*, 5 minutes later, same as *c*. *e*, time in 0.01 second.

Records of figures 4 to 6 reduced about one-half in reproduction.

DISCUSSION. The antagonism between histamine and formaldehyde here discussed bears certain similarities to that shown in the action of these substances on smooth muscle of the gut, demonstrated by Kendall (1927) although the action on this muscle is different. The effective dilutions are of the same order, and the reversibility of the changes by simple washing is at least as great. The action here is apparently a direct one on the muscle, as seems to be the case with smooth muscle. The formaldehyde must eventually penetrate to all the fibers of this muscle, since the final effect is merely an accentuation of the initial effect, and no evidence of surface fibers only being involved has appeared. The formaldehyde contraction does not appear to be a tetanus in the ordinary sense of exhibiting fused separate contractions, and the action potential is at all times apparently a simple sequence consisting of the two typical parts, although certain irregularities appear to indicate that different groups of fibers may go into the contracture phase at different intervals after stimulation (fig. 5, *c*).

In the muscle contraction after formaldehyde, not only is the *form* of the tension curve changed, but the work done (or capable of being done) is increased, quite as in the veratrinized muscle. After histamine, the opposite effect may not appear, for if the duration of contraction is slightly shorter, the amplitude of the tension curve is higher. These curves show (fig. 2, *d*), even with this simple recording method, a form similar to that optically recorded by Fulton (1926) for skeletal muscle, with a sudden release or "angle" at the end of the tension phase. The movements of the muscle are slow enough so that the lever used (15 per second free period) may have followed the tension fairly precisely. The "angle" occurs at a time when lever inertia should have a minimum effect;—that is, after an almost horizontal tension plateau.

The action potentials recorded after formaldehyde show an interesting variability with variation in the strength of stimulus (fig. 5, *e*, *f*). By connecting a non-reactive shunt across the leads to the amplifier, a potential wave of full maximal potential may be reduced in the record to the amplitude of a submaximal potential (i.e., one in which not all fibers participate) with no significant distortion of form. That is, instead of part of the fibers (active) being shunted by the rest (inactive), in a submaximal response of the muscle, all the fibers in activity at once are shunted by an external resistance with approximately the same effect on the potential wave, a reduction in amplitude. We may thus compare the forms of potentials from different strengths of stimulus by superposition of the records. By leading slightly off the stimulating cathode or effectively between cathode and anode (Bishop and Gilson, loc. cit.) the shock distortion has, practically, subsided in the record before the action potential starts.

Under these conditions, when the shock strength is weak, the initial maximum of the potential response may be actually lower than the later por-

tion. As the shock strength increases, the earlier portion of the wave rises relatively more rapidly than the later portion, until it finally rises above it. After the action of histamine no such phenomenon appears, in fact the opposite may occur; that is, the initial crest increases, relatively to its amplitude, as rapidly as the later portion, and probably more rapidly. The fusion of the two portions of the curve here renders quantitative measurements impossible.

This sequence resembles a result previously reported, but not explained, of stimulating the frog sartorius (Bishop and Gilson, loc. cit.). Stimuli of different strengths to the same muscle resulted in differences in the relative amplitudes of the first peak and the subsequent lower portion of the action potential wave. This seems to indicate two more or less distinguishable processes separated in time in normal muscle, a possibility which Gilson (1927) has considered with respect to the normal monophasic electrogram of heart tissue. One of these at least must be capable of responding to different degrees with different stimuli near the stimulated point, in a given fiber. This might be due to the alteration by the stimulus of the local condition of the muscle,—which, to be sure, is the method by which a stimulus stimulates,—but in any case, the question might be raised what the maximal response of the muscle is, and whether the term "all" in the all-or-none law represents more than a technicality. If the muscle element can be made to respond, mechanically or electrically, by a superthreshold stimulus more strongly than it does to a threshold stimulus, then it is conceivable that the "normal" response is not the maximal of which the muscle is capable, but is that intensity of response to which the stimulus from nerve or from the adjacent section of the muscle fiber is capable of exciting it. That is, the mechanical and the late electrical response may *normally* (i.e., by indirect stimulation), never reach the true maximum of which the muscle is capable. This is also suggested by the increased power of response evoked under the influence of veratrin and formaldehyde. These substances must somehow release more energy accompanied by a greater potential, through the contractile mechanism already effective in the normal reaction. At the same time their ability to do so seems to depend upon the strength of the stimulus as affecting the contractile process directly, rather than upon a changed irritability of the muscle.

In this connection may be quoted the findings of Dean (1901) that the direct stimulation of muscle may elicit a mechanical response considerably greater than that from "maximal" stimulation of the nerve. This result might be due to the summation of the effect of the normal irritable process plus the direct effect of an artificial stimulus to the contractile mechanism. Or it might be considered that the effect of the current, like the effect of the drugs discussed above, was to give the muscle a greater "potential"

ability to contract, which is, however, released through the normal irritable mechanism. The two possibilities are difficult to differentiate in practise, and in theory, either compromises the integrity of the all-or-none law in the naïve form in which it is usually stated.

Finally Querido (1927) reports that if a nerve attached to a muscle is treated with veratrin, at a time when the veratrin soaking along the nerve has just affected the muscle, an indirect stimulus may produce a simple response while a direct stimulus calls forth a typical veratrin contraction. Further, Querido presents contraction curves for veratrinized muscle which change in form with change in strength of stimulus, in a manner which parallels the changes in form of the action potentials reported above, from muscles treated with formaldehyde.

Now in the veratrin or aldehyde treated muscle, the *mechanical contraction*, at the height of the effect, takes place in two stages, the usual twitch followed by a prolonged and more or less separate contraction. When this occurs, the potential wave shows a similar form. As the formaldehyde is washed out, the second phase of contraction encroaches upon the first, until the two are indistinguishable (fig. 3, *j*, *k*) and the potential follows suit. The second phase of potential may not appear prominently, much beyond the range of spread of the stimulating current, that is, it is often not conducted at full amplitude.

The *normal action potential* of skeletal muscle recorded near the stimulus appears to consist of two over lapping or summed effects (Bishop and Gilson, loc. cit.), an early spike preceding contraction, and a later portion accompanying the tension development of the twitch; but this second electrical process appears also to be poorly conducted. That is, after conduction of more than 5 to 10 mm. in a parallel fibered muscle the second effect is not distinguishable as such, though a potential still lasts throughout the tension phase. The records of these two effects may show some fusion as processes in fibers with different conduction rates fall out of phase, but the falling off appears to be too great to be explained by this factor alone.

Finally, in the aldehyde treated muscle, these two *normal* electrical phases can be distinguished near the stimulated region, followed by a third which accompanies the typical contracture, in a suitable stage of the chemical's action. Also, in fatigue, the veratrin potential effect passes off first, then the potential during the twitch contraction, and only later is the first spike depressed, as in normal untreated muscles. Or, during recovery, upon washing out of formalin, if the muscle is not fatigued, the potential accompanying contraction may result at a strength of stimulus which fails to produce a noticeable first spike of the potential (fig. 5 *e*).

These observations are consistent with an hypothesis whose premises are as follows: The first spike of the normal muscle action potential may be considered the sign of a nerve-like process conducted (possibly) in an

all-or-none manner, which serves to initiate the complex chain of chemical reactions resulting in contraction of the muscle. The second part of the normal twitch potential serves as a sign of an electrochemical reaction in this chain which underlies or accompanies tension development. It may be all-or-none to the extent that the first process, being normally constant, initiates a constant chain of sequelae along a uniform fiber, but if this effect is augmented by a "supermaximal" stimulus, the resultant events will also be augmented in intensity at least in the region affected by such a stimulus. This is consistent with the findings of Dean (loc. cit.), on the effects of maximal direct as compared to indirect stimulation, and with the facts of the "Tiegel contracture" upon strong direct stimulation.

Under the influence of veratrin-like agents the stages in the chemical sequence which cause tension to be developed are accentuated, both in intensity and in duration, and appear to respond to direct stimuli without the necessary intervention of the normal irritable mechanism, which can even be depressed.

Histamine in skeletal muscle on the other hand appears to intensify the first stage of the process, if it affects it at all, and decreases in duration at any rate the potential. The tension curve shows no sign of a decrease in amplitude, and its angularity might be taken to indicate a more abrupt cessation of activity than normal, as compared with the opposite effect of aldehyde.

These phenomena receive a different interpretation by Verzar (1925), who, although he finds a prolonged potential appearing under the influence of aldehydes in both isotonic and isometric records, states that this potential accompanying contractions is to be assigned to the change of form, and of resistance in the muscle circuit, and not to activity of a muscle irritable mechanism. If this were the case, the ordinary tetanus of skeletal muscle, untreated by aldehyde, should show the same type of prolonged potential plateau as does the tetanic aldehyde treated muscle recorded by Verzar, although a faster rate of stimulation might be required. Further, the potential should increase in Verzar's records as the mechanical responses summated to a maximum. The alteration in relative intensities between the initial twitch potential and the contracture potential recorded by us seems further to rule out such an explanation. Our potential curves were taken from rigidly isometric muscles, led into a vacuum tube grid circuit drawing no appreciable current, and the factor of resistance change should have been at a minimum.

SUMMARY

Tension curves and action potentials from the retractor penis of the turtle treated with formaldehyde show a typical veratrin-like action of this substance, as previously reported by Verzar for frog muscle. Treated

with histamine, the effect is opposite in certain respects, suggesting an antagonism between these substances for skeletal as for smooth muscle, although the effects themselves are different in the two systems.

The increased potential during the twitch, and the prolonged potential accompanying the contracture, under the influence of formalin, appear to correspond with the potential which, near the stimulated region in normal frog muscle, follows the first spike of the action potential and accompanies tension development (Bishop and Gilson).

These potentials accompanying tension development do not conduct at full amplitude, and vary with the strength of the stimulus more or less independently of the first spike of the potential. This suggests that there are distinguishable electrical signs of two phases of the muscle's response; the earlier that of the irritable mechanism corresponding to nerve, the later potentials signalizing the response of the contractile mechanism; the two being fused or overlapped in the normal conducted muscle potential.

If this hypothesis be correct, it amounts to stating that the contractile mechanism of skeletal muscle can be stimulated either by a special irritable mechanism in the muscle, or by an electrical stimulus directly, or by both together, the effects of the two stimuli being summated in the contraction up to the limit of the muscles' ability to respond; the normal mechanical response to an indirect or nerve stimulus being therefore not maximal.

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THE SECRETION OF URIC ACID BY THE FOWL

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During the course of some experiments on the fowl's kidney it was discovered that substances could be easily injected into the kidney, and histological examination showed that the injected substances may even reach the glomerular capsule (fig. 1). These results naturally suggested the possibility of obtaining direct evidence on the question of resorption in the renal tubules. Similar experiments have been attempted on animals but, as Cushny (1) showed, it is not possible to inject fluid directly into the tubules of the mammalian type of kidney owing to a valve effect taking place at the calyces. Recently the question has been raised again under the terminology of pyelo-venous back-flow, and Bird and Moise (2) in discussing this subject state that both india ink and prussian blue may be injected as far as Bowman's capsule, at pressures below maximum secretion pressure.

The first experiments were conducted to measure the secretion pressure of the bird's kidney. This was accomplished by placing a cannula into the ureter and allowing the kidney to secrete until a maximum was reached. These experiments showed that the secretion pressure is very low, the highest recorded in over thirty experiments being only 42 cm. of water. This result was in accordance with our expectations since, as has been shown elsewhere (3), clamping of the ureters most promptly causes the blood uric acid to rise, and furthermore none is stored in the kidney.

Clearly in order to inject substances into the kidney a greater pressure than the secretion pressure would be necessary. Attention being directed to this subject it was elicited that in an absolutely fresh preparation as a rule it was difficult to inject fluids into the kidney without using such pressures (100 cm. water or even more) that suspicion of mechanical damage to the kidney being aroused; on the other hand after a certain time, and especially if the kidney had been secreting against resistance, the pressure required was less than that which may be developed by the preparation itself. A second point also noted was that once fluid commenced to enter the kidney it would often continue to do so under a lower pressure, but that if the preparation be allowed to secrete freely for a time, the inflow would not recommence at this low pressure. Bird and Moise also note this di-

minished inflow resistance but they do not state if it is permanent or not. In interpreting these results it is clear that two distinct factors are involved. First, the secretion pressure of the kidney, and secondly, the effect of ureteral movements. The results of some experiments on the latter have been reported (4); they may be briefly summarized here by saying that the ureteral movements are such that a downward milking effect may be produced so that a difference of pressure of up to 40 cm. of water may be occasioned between the upper and lower ends. As these experiments involved some damage to the ureter even greater pressures might be conceived in an intact bird. It was also noted that as the ureter became fatigued, by working against pressure for a time, this milking effect became greatly impaired or even abolished.

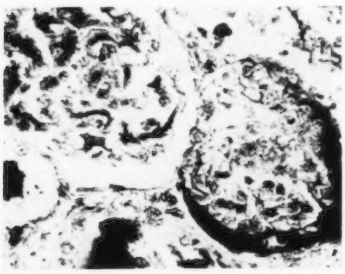

Fig. 1. The untouched photomicrograph of a section of a fowl's kidney injected with prussian blue jelly per ureter, and carmine jelly by the renal artery. Two adjacent glomeruli are shown. The one is surrounded by a dark mass of prussian blue, while the other capsule contains none.

These experiments naturally threw light on the subject of inflow resistance; they also show that the secretion pressure of the bird's kidney is very low indeed, since that measured by placing a cannula in the ureter will be largely due to the milking effect. From the various data available this would appear to be between 10 to 20 cm. of water, that is to say, a pressure comparable with the biliary secretion pressure (5), and far less than the pressures recorded in the mammal (6).

Two factors are concerned in the effective secretion pressure, namely, the true secretion pressure, both glomerular and tubular, balanced by the permeability, or absorbability, of the tubules. It is, for example, conceivable that the glomerular filtration pressure may be 40 to 60 mm.Hg below that of the blood pressure, say 40 mm. Hg, (54 cm. water) as is suggested for the mammalian kidney, but that the tubules offer such little resistance to fluids passing inward to the blood stream that this "head" be undetectable by our experimental method. The extreme ease with which uric acid is dammed back in the blood, and other evidence (3.7) while suggesting most strongly the presence of tubule secretion, showed this to take place at a very low pressure, and thus the main secretion pressure was expected to be due to filtration. Efforts were therefore directed to ascertain the effects of blood pressure changes on the secretion pressure. These experiments were carried out before the data became available on the effects of the ureteral movements, which complicate the interpretation, but in the light of our present knowledge on these move-

ments, the results become reasonably clear. They were conducted in the following manner.

METHOD A ureteral cannula having been inserted, it was connected to the apparatus illustrated in figure 2. This consisted of a manometer,

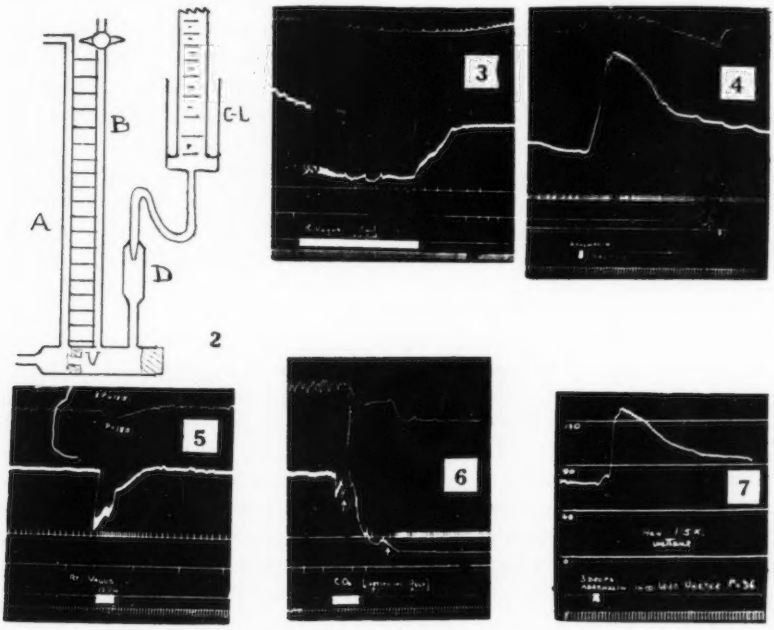

Fig. 2. Diagram of the apparatus used to measure secretion pressure and inflow resistance.

Fig. 3. Shows the effect of stimulating the vagus on the inflow into the ureter. Upper tracing ureteral; second, blood pressure; third, inflow and base line; fourth, urine secretion of the other kidney. Signal and time tracing. All time tracings are 5-second intervals.

Fig. 4. Shows the effect of adrenalin. Tracings as before except the inflow curve is the lower one, the upper being the secretion from the other kidney. The line between the two is the base line.

Fig. 5. Shows the effect of vagal stimulation on the rate of inflow. (Same as fig. 3.)

Fig. 6. Shows the effect of killing the bird on the inflow and ureteral movements. (Same as fig. 3.)

Fig. 7. Shows the results of an injection of adrenalin per ureter. Pressure used 36 cm. of water.

A, which was separated from the rest of the apparatus by a very delicate valve, *V*. To the top of *A* was connected a sensitive recorder. Any urine secreted was therefore confined to the anterior part of the apparatus,

and could be directly measured. In order to measure inflow resistance a constant level, *C-L*, apparatus was connected to the manometer *A*, the fluid from this being measured by passing through a special drop recorder, *D* (8). For convenience a second manometer *B*, was added so that the inflow pressure could be quickly and accurately adjusted, since the interposition of the recorder upset the levels. While inflow was taking place this manometer was closed at the top, so that any fluid entering the kidney must necessarily pass through the recorder. With this apparatus ureteral movements, increase and decrease of secretion pressures, and the actual inflow at any desired pressure are recorded.

Results. Adrenalin, which raises the blood pressure, frequently increases the secretion of urine and stimulates ureteral movements, not infrequently increases the secretion pressure (see fig. 4). The whole of this effect could be accounted for by the increase of secretion and the known effect on the ureter, it is however possible that the filtration pressure is actually higher; as however the effect of adrenalin on the secretion pressure is by no means constant, and as it always produces an increased blood pressure, it does not appear that this effect is very marked, and probably only amounts to 1 to 2 cm. of water. Similarly ergotoxin may produce comparable results. With this drug the blood pressure effect is more prolonged, and there may be quite a marked effect on the ureters. Procedures which lower the blood pressure sometimes lower the resistance to inflow. Clear-cut results could be obtained by a prolonged vagal stimulation, by which most interesting results were obtained (fig. 3). As will be seen after the blood pressure has fallen for some time, the inflow fluid commences to run, and in this experiment continued to do so even when the stimulation had ceased, though at a slower rate. That this is not merely due to a filling up of a dilated ureter, which will hold 1 to 2 cc., is shown by the fact that the inflow continues far beyond this amount, also in preparations in which the inflow has already been started the rate is increased (fig. 5). It is highly probable that the secretion pressure has been diminished by this procedure, for it was shown elsewhere (7) that stimulation of the vagus causes marked slowing of the renal circulation, besides the fall in blood pressure. Something however must have happened to the ureter, since it is no longer able to prevent fluid passing back along it. Does this show that the vagus has an influence on its movements? As was stated in another paper (4) clear-cut evidence on this point could not be obtained, and the explanation appears to be that the loss of tone in the ureter is due to the poor circulation. That such an effect really does take place is shown in figure 6.

This experiment shows beyond reasonable doubt that the passage of fluid into the kidney takes place without actual mechanical damage since it can be achieved at a pressure less than that which the bird can develop itself, and with no direct interference with the kidney.

Further experiments with this method which involved the use of various drugs showed the importance of the ureteral movements to so outweigh the renal factors as to render these latter impossible of accurate interpretation. Efforts have been made to eliminate the ureteral movements by the use of drugs, or by the insertion of tiny "suction" catheters. Up to the present however these experiments have not been satisfactory enough to report.

As was mentioned previously, once the inflow commences it frequently continues, and gradually increases its rate, or continues at a lower pressure. The full explanation of this fact is not known, but it is significant that if such a preparation be allowed to secrete freely against zero pressure for a little time, the inflow will no longer take place at the same pressure. This is extremely suggestive of an action on the ureter since it is possible to restore "milking" movements which have been abolished by causing them to work against pressure by means of a short rest.

In view of this difficulty another attempt was made to circumvent the function of the ureter, or at any rate to minimize its activities, by an analysis of the action of drugs on the rate of inflow. This was started either by vagal stimulation, or allowing the ureters to become fatigued by working against resistance for some time, usually about one hour.

Results. Adrenalin and ergotoxin impair, or even stop depending on the particular conditions of the experiment, the inflow (fig. 4). Vagal stimulation, histamine, pituitrin, amyl nitrite and CO_2 all increase the rate of inflow (figs. 5, 6). Furthermore under good conditions killing the animal by some such procedure as inhalation of CO_2 will start and maintain inflow (fig. 6). This result is mainly due to paralysis of the ureter and partly to diminution of renal resistance. This is shown by the fact that such a procedure in an animal in which a good inflow is taking place may not have very much effect.

A few experiments were directed to ascertain if there was any correlation between the renal venous pressure and inflow. The renal venous pressure is readily measured by simply putting a cannula in the femoral vein since this connects directly with the renal veins. The normal pressure is around 4 to 6 cm. water, and varies with the conditions of the circulation, dropping perhaps to 2 cm. or rising to 10 cm. The lowest inflow pressures are rarely below 10 cm. water, and more often are between 17 and 20 cm. Thus they are always larger than the venous pressure. It is quite conceivable that some relation exists between the rate of inflow and renal venous pressures, but the method used is not sufficiently sensitive to demonstrate any such relation.

As was previously mentioned, fluids enter the kidney in far greater amounts than can be accounted for by mere filling of the ducts and tubules, and the question arose as to where this fluid was going. Was it merely diffusing through the kidney into the abdominal cavity, which it could readily do since the ureteral branches, or tubules connection, run straight

through the kidney to the surface, and excessive pressure suddenly applied can cause a rupture at this point. The bird's kidney is also extremely friable and easily subject to injury. This question is readily decided by perfusing into the kidney drugs having a characteristic action. when it is readily demonstrated that such bodies promptly enter the blood stream (fig. 7). It is true however that after a large quantity of fluid has been perfused into the kidney the abdominal cavity may contain free fluid at the end of the experiment, and this led to a very thorough test to see if this was due to leakage. Such tests however showed that simple leakage, or even direct diffusion was not the usual cause of this free fluid, which only occurred when large amounts had been perfused; also such fluid is found if large amounts are injected intravenously. Any drug that has been examined can be shown to enter the blood stream with great ease by the ureter route. The list includes adrenalin, pituitrin, histamine, nitrite, atropin and methylene blue. With this latter substance one can readily show its absorption by one kidney and in a few minutes its excretion by the other. Post-mortem examination in such cases shows no staining of the abdominal cavity, unless large amounts were used.

Attention was then directed to the question of selective resorption. Did the kidney offer greater resistance, or fail to absorb some bodies in contrast to others? Experiments were conducted with Ringer, distilled water, uric acid (1 per cent in 1 per cent hexamine), hexamine (2 per cent), urea (1 per cent), and sodium sulphate (10 per cent) solutions.

Protocols showing the rate of perfusion

Figures given are cubic centimeters per ½ hour

	Amount	Perfusion pressure Cm. water	Substance
Expt. 1	2.0	23	Ringer
	5.0	23	Ringer
	18.0	23	Ringer
	11.5	23	Ringer
Expt. 2	4.5	36	Ringer
	6.0	36	
	15.5	36	
	2.5	23	
Expt. 3	3.2	23	Ringer
	5.7	23	
	4.1	23	
	5.0	23	
	17.8	23	Urea 1 per cent
Expt. 4	2.45	23	Urea 1 per cent
	15.2	23	Urea 1 per cent
	27.8	23	Dist. water

Protocols showing the rate of perfusion—Concluded

	Amount	Perfusion pressure Cm. water	Substance
Expt. 5	7.0	19	Ringer
	16.0	19	
	16.0	19	
	16.0	19	
	8.0	19	Sod. sulphate 10 per cent
	15.5	19	
	14.0	19	
	14.0	19	
Expt. 6	9.0	23	Ringer
	18.3		
	12.0		
	15.0		
	12.0		
	10.3		
	12.0		Sod. sulphate 10 per cent
	2.4		Uric acid 1 per cent in hexamine 1 per cent neutral
Expt. 7	11.4	35	Ringer
	14.9	26	
	5.6		Uric 1 per cent in hexamine 1 per cent
	5.6		
	28.6		Ringer
Expt. 8	3.0	36	Ringer
	4.0		
	1.7		Uric 1 per cent in hexamine 1 per cent
	1.5		
	9.3		Ringer
	3.5		Hexamine 2 per cent
Expt. 9	3.4	36	Ringer
	5.4		
	3.2		Hexamine 1 per cent
	4.9		
	4.2		Uric 0.5 per cent in hexamine 0.5 per cent
	3.3		Uric 0.5 per cent
	10.8		Ringer
	17.8		
Expt. 10	1.0	23	Ringer
	2.0		
	1.5		Hexamine 2 per cent
	4.5	36	
	0.6		Uric 1 per cent in hexamine 1 per cent
	1.7		
	9.5		Ringer

See also figures 8 and 9.

Notice that there is practically always an increase in perfusion rate as the process continues. Thus the apparently sudden increase in experiment 3 with urea should

be regarded with suspicion. Also whenever there has been a change made from one fluid to another there is frequently an alteration, usually a decrease. An increase may have more significance. This is well shown in experiment 4. All fluids were approximately at body temperature. The uric acid solutions were made by dissolving uric acid in hexamine and neutralizing with soda.

These experiments show that with the exception of uric acid all the substances tried readily pass through the kidney by this route. Uric acid in one experiment (Sept. 9) also showed no great difference. I have not made comparative studies on the diffusibility of these various substances, partly because the results obtained with simple membranes would have very questionable value, since this diffusion takes place through living tissues, and, as will be seen from a comparison of the effects of Ringer and uric acid, this latter substance is much more poisonous to the kidney. Furthermore these results offered no support for a real selective mechanism since even uric acid enters the kidney at a fair rate.

Naturally in regard to these experiments the question arises is the kidney damaged by this perfusion? As was shown elsewhere (3), prevention of the renal secretion by clamping the ureter, thus offering resistance, may lead eventually to damage, but that this only takes place after the lapse of many hours. Thus if the kidney shows evidence of damage, by inability to excrete uric acid, after only a short perfusion it can be taken as evidence of damage due to the perfusion, and not merely due to the pressure (3).

To answer this question experiments were conducted in which first the one ureter was perfused, then after a varying period the other ureter would be perfused while the first one was allowed to take over secretion. In every experiment as the result of this procedure the blood uric acid rose, showing that the kidney was damaged. In some cases the actual amount of uric acid excreted did not alter from hour to hour. These results are illustrated very well by figure 8 which is the data obtained from one of these experiments in which Ringer alone was used as perfusing fluid. A study of this graph brings to light a very interesting series of facts. First, that perfusion of one kidney alone makes no significant changes in either the uric acid excreted or the blood uric acid. That immediately the perfused kidney takes over the excretion of this substance its blood percentage goes up, but that the total amount is unchanged. Along with this will be noted a marked increase in urine, the result on the concentration of uric acid being markedly to lower it. It is very difficult indeed to explain these figures on the filtration resorption basis, the obvious explanation being that perfusion of the kidney has slightly damaged it so that it is unable to excrete uric acid so efficiently, but the filtration of water is unimpaired, or even rendered greater, though in this connection the amount of fluid perfused into the bird would naturally tend to cause a diuresis. This suggestion is supported by other experiments In which either longer

periods of perfusion or other substances, such as uric acid solutions, are used. As will be seen from figure 9, immediately the right (perfused) kidney is set to work the blood uric acid rises, and the output falls, in spite of the fact that there is in this case an actual increase of urine. This increase is not constant.

These experiments, although proving that absorption can take place from the tubules, offer no support to the theory that this is a normal mechanism, since even such harmless substances as Ringer cause obvious damage to the secreting mechanism. Furthermore if one calculates the various amounts involved this conclusion is strongly supported. Taking the data from figure 8 it will be seen that at the third and fourth hours

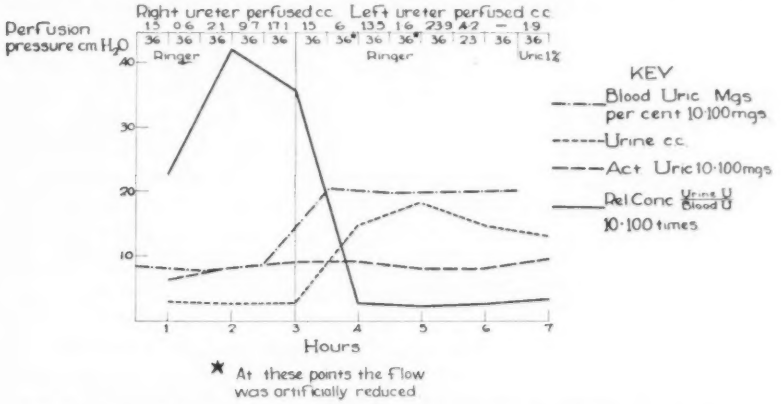

Fig. 8. Curves drawn from an experiment showing slight damage to the renal mechanism by perfusion. Note in this and in figure 9 the uric acid estimation of the blood was made ½ hour preceding the urine examination. This makes the blood uric acid curve apparently rising before the change is over.

respectively the blood uric acid rises from 8.7 mgm. to 20.8 per 100 cc., the actual uric acid drops from 87.7 to 87.0 mgm. and the urine rises from 2.8 cc. to 15.0 cc. Calculating the relative concentrations shows a fall from 360 to 28. Assuming a pure filtration resorption mechanism it will be seen that at the third hour 1010 cc. of blood filtrate are required, of which 1007.2 are resorbed, namely, 99.8 per cent, while at the fourth hour only 418 cc. of filtrate are required of which 403 are resorbed, or 96.4 per cent. In other words, with less work to do the resorption machinery becomes less efficient. Similarly an examination of the results shown in figure 9 illustrates the same fact. That this is not a simple water diuresis is shown by the rise of blood uric acid. Furthermore it will be seen that during the first three hours of perfusion approximately 2826 cc. of filtrate

would have been required to excrete the uric acid, of which 2817 are resorbed, (99.5 per cent) yet the addition of only 31 cc. (1.2 per cent) by perfusion has been sufficient seriously to disturb the renal mechanism.

That this result is not simply due to a back-pressure effect is shown by examining the renal function of kidneys which have not been allowed to secrete for 6 to 8 hours, by clamping the ureter. In these experiments release of the pressure promptly allows the kidney to take up its function. It is true however that such a procedure will eventually damage the kidney (3).

Discussion. In the series of papers dealing with the excretion of uric acid by the fowl evidence has been accumulated which makes the application of Cushny's theory of renal secretion to the bird's kidney almost impossible.

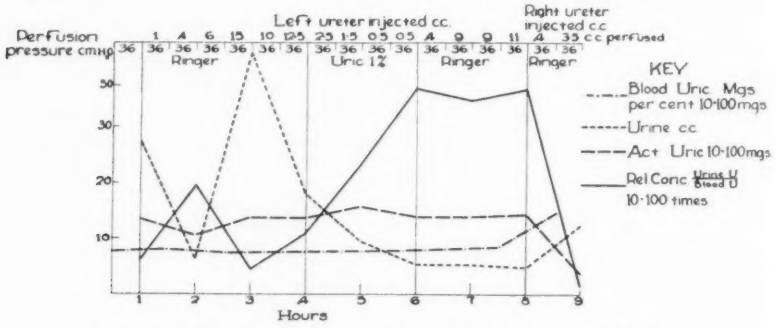
Left ureter injected cc.
Right ureter injected cc
Perfusion pressure cm Hg
1 4 6 15 10 12.5 2.5 1.5 0.5 0.5 0.5 4 9 9 11 4 3.5 cc perfused
36 36 36 36 36 36 36 36 36 36 36 36 36 36 36 36 36
Ringer
Uric 1%
Ringer
Ringer
KEY
Blood Uric Mgs per cent 10-100 mgs
Urine cc
Act Uric 10-100 mgs
Rel Conc Urine U / Blood U 10-100 times
50
30
20
10
1 2 3 4 5 6 7 8 9
Hours

Fig. 9. Shows the effect of greater damage to the kidney by perfusion

First we have the evidence of Mayrs, to whom belongs the credit of suggesting specific uric acid secretion in the bird as opposed to the modern filtration resorption theory. Mayrs showed that uric acid is concentrated to a much greater degree than any other urinary substance examined, which he concluded either means that everything else is resorbed, or as a much more probable explanation, that uric acid is actively secreted. Mayrs (9) gives as the highest concentration of uric acid achieved by the kidney during his experiments (table 11, p. 280) a $\frac{\text{urine}}{\text{plasma}}$ ratio of 62.7. In his experiments, however, diuresis was usually produced and consequently he was dealing with a dilute urine. If some of the results of my experiments over longer periods be examined it will be seen that Mayrs' figures become almost modest, and that uric acid can be concentrated to a really remarkable degree.

Experiment, November 17, 1925

BLOOD URIC	URINE URIC	RATIO	(HOURLY PERIODS)
per cent mgm.	*mgm. per cent*		
6.2	598	96.5	An experiment on the effect of pituitary
6.7	2,760	412	
7.3	4,140	570	
8.1	17,500	2,160	Pituitary period
7.0	21,600	3,110	Pituitary period
6.1	8,700	1,425	Pituitary period
7.1	3,820	540	
7.3	3,840	527	

His arguments therefore become still more potent, and indeed per se are very strongly in favor of uric acid secretion.

To this positive evidence we have added the facts that the renal blood flow and uric acid secretion are independent both in normal birds, and under the influence of pituitary. In the former the blood flow steadily fell while the removal of uric acid from the blood was rapidly increasing (7); in the latter the blood flow is known to be considerably less (7) yet actually more uric acid was excreted (3). This evidence is definitely against the filtration-resorption theory. Then we have the evidence that water and uric acid behave independently. This is shown in the blood flow experiments, remarkably so under the influence of pituitrin, and in a less conclusive manner under atophan. Also the experiments on perfusion shown in this paper indicate that the uric acid mechanism is damaged while that of water elimination is clearly less affected since actually more may be excreted.

Added to this strong evidence in favor of the secretion of uric acid, and its dissociation from the water elimination, is the fact that although absorption from the tubules can undoubtedly take place, no real selectivity exists; also it seems to be an abnormal mechanism in that small quantities of even non-poisonous fluids such as Ringer's solution when perfused into the kidney rapidly damage it. Furthermore, fluids of all kinds pass most readily through the kidney into the blood stream, including even such bodies as adrenalin which might be supposed to hinder its own absorption.

Then there exists the evidence of at least three workers (3, 9, 10) that with a diuresis the uric acid concentration may actually be increased which is the reverse of the effect found on perfusing (i.e., damaging) the kidney, and is best explained by a secretion of uric acid.

We have also the evidence of Mayrs that secretion against pressure affects but little concentration of the urine, to which I have added the fact that serious obstruction most promptly stops the excretion of uric acid, which merely remains in the blood stream. This indicated, and was confirmed

by direct experiment, that there is no storage in the kidney under these conditions, nor does the kidney substance contain a concentration of uric acid as high as an equivalent amount of urine.

It will be seen therefore that the usual arguments do not apply to the bird's kidney. This is well illustrated by a critical examination of the experiment published elsewhere (7). In this experiment the renal blood flow was measured directly, as was the urine output, also analyses of the uric acid concentrations of the bloods entering and leaving the kidney were made. Taking the figures at the beginning and the end of the experiment we find that

	BEGINNING	END	PER CENT DIFFERENCE
Blood-flow (cc. per min.)	82.5	65.0	21 (d)
Urine (drops per min.)	8.0	2.5	69 (d)
Arterial uric (mgm. per 100 cc.)	14.1	21.7	
Venous uric (mgm. per 100 cc.)	14.1	15.1	
Percentage removal	Nil	30.5	

Assuming the filtration-resorption mechanism we first notice that the blood flow falls 21 per cent while the urine flow diminishes 69 per cent. To explain this fact we must assume either a change in glomerular permeability or a greatly increased resorption of the tubules, amounting to 252 per cent either way. (In this calculation, allowance is made for the decreased filtration resulting from diminished blood flow.) To these figures however must be added the change in the uric acid, which may be taken to be excreted since there is no storage in the renal tissue. This amounts to a total quantity of 4.3 mgm. $\left(\frac{30.5}{100} \times \frac{65}{100} \times \frac{21.7}{100}\right)$ per minute at the end, being nil at the beginning of the experiment. This amount of uric acid would require 20 cc. of filtrate of which 0.08 cc. (2.5 drops) remained unabsorbed. If these amounts represent a simple change in filtration rate then the glomerular permeability has altered 75 times $\left(\frac{30}{8} \times \frac{20}{1}\right.$ note; 30 drops equal 1 cc.). If no filtration change has taken place, then we must assume that either the glomeruli were impermeable to uric acid at the beginning, or that this was absorbed by the tubules. That the glomeruli be impermeable to uric acid is unlikely, since Mayrs has shown, and which I have confirmed, uric acid in serum quantitatively filters through colloidin membranes. We are thus faced with accepting either a permeability change of 75 times, or a resorption of the most important waste product. I venture to think that neither choice is a very happy one, and neither fits into the reasonable mechanism propounded by Cushny.

Bearing in mind all the facts which have been brought forward we must

conclude that the only understandable mechanism to explain them is an active secretion of uric acid, with an independent mechanism for water excretion; probably by filtration, since the water excretion seems roughly to follow the blood flow and pressure. It would also seem probable that the filtrate would contain filterable substances. In view of the doubt thrown on the existence of a normal tubular absorption by the perfusion experiments, it would appear probable that the glomerular permeability does change quantitatively, but without definite evidence it would appear unwise to postulate any specific change to any given substance, though it is true that a decrease of permeability might affect a substance just on the borderline to such an extent that it would no longer filter through, the glomerulus still remaining permeable to water.

While evidence is accumulating for other kidneys which support secretion, it cannot be over-emphasized that the bird's kidney is so peculiar that direct analogies cannot safely be drawn.

In coming to the above conclusion, however, it appears necessary to define what the word secretion is intended to mean. Clearly the secretion of uric acid is not a process akin to salivary secretion, which involves storage and even construction of the secreted bodies, which the cells can eject against very considerable pressure. Certainly uric acid does not simply filter through the renal cells as through a colloidin membrane, since the process requires the intervention of energy as a concentration of over 3000 times suggests, and which is also shown by the fact that asphyxia promptly stops secretion, and this may be occasionally shown in a very marked manner.

We have however a beautifully analogous case in the secretion of bile. This substance is taken up from the blood stream in a specific manner by the liver cells, and then gently pushed out on the other side. The amount dealt with in this way depends on the amount in the blood stream; (Babkin, p. 70) back pressure promptly interferes with this mechanism, the bile piling up in the blood stream in the same way as uric acid does in the fowl when the renal mechanism is affected. Also like the fowl's kidney no storage takes place. It is suggested therefore that the process of uric acid secretion in the bird is by a process involving a similar type of mechanism to that part of biliary secretion which picks up the bile from the blood, though of course this is modified to suit the different substance. Such a mechanism would satisfy all the data obtained in a long series of experiments on this subject.

CONCLUSIONS

1. Fluids of all kinds can readily enter the bird's kidney from the ureter at iow pressures. They may go as far as Bowman's capsule.

2. True selectivity in the absorption of such fluids has not been demonstrated.

3. Perfusion even for a short time injures the uric-acid-secreting mechanism, and suggests that tubular absorption is not a normal mechanism, though may unquestionably take place.

4. The secretion pressure of the bird's kidney is between 10 and 20 cm. water pressure.

5. Conclusion drawn from these and other results already published is that the bird's kidney actively secretes uric acid.

6. That this mechanism is distinct from that involving water excretion.

7. A mechanism analogous to biliary secretion is suggested as one which might deal with the uric acid, while it is probable that water is filtered by the glomeruli.

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THE EFFECT OF DAILY TRANSPLANTS OF THE ANTERIOR LOBE FROM GONADECTOMIZED RATS ON IMMATURE TEST ANIMALS[1]

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Certain points regarding a reciprocal pituitary-gonadal relationship have been discussed previously by Smith and Engle (1927) and Engle (1928). As another step toward an understanding of this mechanism, it was necessary to determine the gonadal-stimulating potency of the anterior lobe of castrated animals.

The histological condition of the pituitary of the castrated rat has been described by Addison (1917), and the literature dealing with many species recently reviewed by Lehmann (1927). In the rat at least, castration is characterized by a progressive change in the cell content of the pituitary, resulting in a condition which may be called terminal some time between the fourth and seventh month after castration (Schenck, 1927). At this time, the exact temporal relations of which have not been determined, the pituitary has a greatly increased number of basophile cells, the largest of which are extensively vacuolated.

Similar castration effects on the pituitary are reported after x-ray sterilization of the male (Schenck, 1927). The reciprocal mechanism has further been tested by Lehmann (1927), who states that a restoration of the oxyphile cells of the pituitary occurs after the injection of the follicular hormone into spayed rats.

The response of immature rats and mice to fresh transplants of anterior lobe from normal animals has been established by Smith (1926) and Smith and Engle (1927). By applying the method of daily transplantation of the fresh anterior lobe devised by Smith (1926), it has been possible to determine the effect of bilateral gonadectomy on the secretion of the gonadal-stimulating factor of the anterior lobe.

EXPERIMENTAL. The experimental group of animals consisted of 62

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[2] The experimental work for this publication was done in the Laboratory of Anatomy, Stanford University.

rats of both sexes. One group of 37 males and females was gonadectomized at ages ranging from 20 to 30 days. This constituted the prepubertal group. The second experimental group was composed of 25 adult animals of both sexes which were over one year of age when the gonads were removed. The females of this group had all previously been mated, and had reared litters. Some of the males, but not all, had sired litters.

Both the prepubertal and adult gonadectomized groups lived for eight to nine months after removal of the gonads. Following this period, eight to nine months after castration, these animals were sacrificed and the pituitaries were transplanted on successive days into immature mice and rats, the test animals. Each group of test animals consisted of littermate sisters, the mice being 17 days of age at the first treatment, except two which were taken at 19 days. The rats were given their first treatment on the 22nd or 23rd day of life. The mice received approximately one-half of the pituitary of the rat, while the entire gland was injected into the test rats. In addition to the untreated litter-mate controls, one animal in each of the test groups received the gland from a normal, non-gonadectomized rat, thus affording a further control in each test group. Specimens of the hypophysis from both the adult and prepubertal castrated groups and their normal litter-mates were fixed and prepared for microscopic study. The test animals were observed for the establishment of the vaginal orifice which, for this work, is a valid sign of sexual maturity. The first group of rats shown in table 1 were litter-mates, 22 days of age at the first treatment, and were treated on five successive days. The first received the hypophysis from normal adult males; the second from males castrated when adult, the third being the untreated animal, and the fourth given the hypophysis of a litter of male rats castrated on the 27th day of life. In this litter as in all the others, autopsies were performed the day following the establishment of the vaginal orifice in any of the test animals.

In every case the rats or mice which had been given a series of transplants from gonadectomized animals showed the establishment of the vaginal orifice earlier than their litter-mates which received transplants taken from normal animals.

The difference in the weights of the ovaries is more marked than the difference in the time of maturity. The ovaries of the test rats which had received hypophyseal transplants which were taken from gonadectomized animals are from four to nine times as great in weight as the ovaries from animals which received transplants from normal animals. The weights of the ovaries of animals receiving the transplants taken from normal animals are quite similar to those from animals of the same weight and length of treatment previously published (Smith and Engle, 1927, table 1). The sizes of the ovaries of immature animals are larger than any previously obtained, age of recipient and length of treatment being the same. There

is more difference between the weight of the ovaries of animals receiving glands from gonadectomized donors and those which were given glands

TABLE 1

Gonadal-stimulating action of the anterior lobe from spayed and castrated rats

NUMBER OF ANIMAL	EXPERIMENTAL		CONTROL		PITUITARY TRANSPLANTS		AUTOPSY DATA			
	Age	Weight	Age	Weight	Donor	Number	Age	Weight	Weight of ovaries	Weight of uteri
Rats										
	days	*grams*	*days*	*grams*				*grams*	*mgm.*	*mgm.*
W2166	22	35			1 Normal ♂	5	27	48	46.8	153.5
W2167	22	34			1 Gonadectomized after maturity ♂	5	27	47	252.4	129.9
W2168			22	36		0	27	50	14.1	21.7
GH2169	22	33			1 Gonadectomized before maturity ♂	5	27	46	268.8	132.5
BH2170	22	36			1 Normal ♀	5	27	48	27.9	85.1
BH2171			22	40		0	27	51	13.3	34.2
BH2173	22	38			1 Gonadectomized after maturity ♀	5	27	52	207.5	112.2
BH2174	22	35			1 Gonadectomized before maturity ♀	5	27	47	190.1	91.1
W2175			23	46		0	26	52	18.1	34.3
W2176	23	44			1 Gonadectomized before maturity ♂	3	26	50	109.9	121.8
W2177	23	38			1 Normal ♀	3	26	44	20.9*	134.8
W2178	23	38			1 Gonadectomized after maturity ♀	3	26	42	106.9	113.1
W2179	23	42			1 Normal ♀	3	26	50	26.6*	182.1
Mice										
M830	19				½ Gonadectomized after maturity ♀	2	22	12	15.1	37.3
M831	19				½ Normal ♀	2	22	11	9.0	63.8
M832	17				½ Gonadectomized after maturity ♀	2	20	12	24.0	44.2
M833	17				½ Normal ♀	2	20	10	8.1	39.1
M834	17				½ Gonadectomized before maturity ♂	2	20	11	10.8	61.6
M835			17			0	20	7.5	3.7	6.7
M836			17			0	20	8.0	3.3	9.8
M837	17				½ Gonadectomized after maturity ♀	3	20	10	11.3	75.0
M838	17				½ Gonadectomized after maturity ♀	3	20	10	15.4	47.6
M839	17				½ Gonadectomized before maturity ♂	3	20	9	20.0	43.0
M840	17				½ Normal ♀	3	20	10	8.0	77.2
M842	17				½ Gonadectomized before maturity ♀	3	21	10	10.2	69.8
M843	17				½ Gonadectomized after maturity ♀	3	21	10	4.5	98.2
M844	17				½ Gonadectomized after maturity ♂	3	21	10	10.2	74.4
M845	17				½ Gonadectomized after maturity ♂	3	21	10	12.1	51.7
M846	17				½ Gonadectomized before maturity ♀	3	21	12	9.1	86.1
M847	17				½ Gonadectomized before maturity ♂	3	21	12	9.0	48.3
M848	17				½ Normal	3	21	12	15.3	45.2

* These two animals having received only three treatments were not sexually mature at autopsy.

from normal animals, than there is between the latter and their untreated litter-mate controls.

Among the mice there is one exception (M 848) in which, although the animal received transplants taken from normal animals, the ovarian weights are greater than in the litter-mates which received transplants from gonadectomized males. For some reason not known at the present the ovarian response of the test rats is relatively greater than that of the mice.

As previously reported (Smith and Engle) the uterine weights are not directly correlated with the ovarian weights, as the great weights of certain uteri consists largely of the uterine fluid, which is a transitory phenomenon. Hence the variability of uterine weight is inconstant, unusually great weight depending upon the autopsy having been done at the optimal time. This is impossible when all animals of a litter are autopsied within the same relatively short period. The data in this small series are insufficient to afford much evidence as to any difference in potency of the glands between the sexes, or between the transplants taken from the group gonadectomized before puberty and those from which the gonads were removed later in life. There appears to be, in fact, no significant difference, although a slightly greater ovarian response may result in those animals which have received the pituitary from animals gonadectomized in adult life, regardless of the sex of the donors.

Discussion. The data here recorded indicate that the anterior lobes of rats which have been castrated for eight months contain a greater supply of the gonadal-stimulating hormone than occurs in the normal animal. The evidence from which this view is derived is the earlier sex-maturation of the test animals receiving this treatment and the increased size of the ovaries, due to an excessive number of large follicles.

It is well known that the hypophysis of the gonadectomized animal is larger and heavier than the normal animal. The present data show that there is a greater quantity of the gonad-stimulating hormone resident in the gland. This may be due either to a greater rate of secretion within the cell after gonadectomy, or to a storage of the hormone after its elaboration within the cells of the gland. Either hypothesis may be considered. The condition of the basophile cells, which increase greatly in size, with the formation of large vacuoles and relative diminution of cytoplasm suggest intra-cellular storage of this hormone. The increase in the number of basophile cells need not signify an increased rate of secretion. Rather it seems that as the cytoplasm of the basophile cell becomes vacuolated, it becomes a storage chamber, and other cells develop from the generalized reserve cells, and maintain the secretion of the organ.

The increase in size and number of the cells following castration, and the change in the histological picture of the pituitary during and after pregnancy show clearly that the genital system has a pronounced influence on the anterior lobe. The present experiments show a definitely greater amount of hormone in the pituitary of the gonadectomized animal than

in the normal, when both are subject to the same test conditions. In other words, as it now appears, the anterior lobe continues to elaborate the gonad-stimulating hormone even after castration, but a considerable amount of the product, if not all, is kept within the gland, probably with the cell of origin. No attempts have been made to test the blood of the gonadectomized rat for this hormone in the circulation or in the urine. Hence it is not known whether it is released into the blood in reduced quantities or whether it is retained entirely in the organ.

As was previously shown (Smith and Engle, 1927), the anterior lobes of immature mice will induce sexual maturity in litter-mate sisters if introduced by the daily fresh transplant method. There is thus shown to be sufficient hormone within three of these glands to induce sexual maturity when transplanted, but will not do so in situ. This and other considerations led us to apply the concept of a releasing mechanism to this organ.

Such an assumption is also indicated to explain the increased biological potency of the anterior lobe of gonadectomized rats. As previously stated, elaboration of the gonadal-stimulating hormone continues, possibly at a lowered rate and much of it is retained within the gland. The condition required for this retention is the absence of the gonad, for in the normal presence of the gonad, secretion and liberation of the hormone progress with less storage than is found in the gland of the gonadectomized animal.

Due to the greatly increased numbers of the basophile cells and their great size and pronounced vacuolation following gonadectomy, and the increased physiological potency of the gland containing these cells, one is led to suspect that it is the basophile cell which elaborates the gonad-stimulating factor of the anterior lobe, although definite statement to this effect seems unwarranted at the present time.

Corroborative evidence is found in the statement of Rasmussen (1921) that the basophile cells are more abundant in the hypophysis of the marmot during oestrus.

SUMMARY

The ovarian response of immature mice and rats following the daily transplantation of the fresh anterior lobe taken from gonadectomized rats is significantly greater than the response to anterior lobe transplants from normal animals.

There is no sex difference in the biological potency of the glands of gonadectomized donors, and no significant difference as regards the age of the donor at the time of gonadectomy.

While the pituitary of the gonadectomized animal is larger than that of the normal, the ovarian response to the former is so great that storage of the gonadal-stimulating factor is thought to occur.

These data offer further evidence which points to a gonadal-pituitary "releasing mechanism," the presence of the gonad being the prime factor involved in the release of this hormone from the organ of origin.

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WEIGHT RELATIONS IN THE RABBIT'S BRAIN

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The present study was undertaken to discover a satisfactory norm for measuring changes in the weight of the brain and in various portions of the brain induced by experimental conditions. In a previous paper (1) we expressed the view that physiological processes in the brain—and we believe this to be true of the other organs—are affected by variations in the water content of the organ. Weed and McKibben (2) showed that a decrease in the size of the brain is effected by an intravenous injection of hypertonic salt solutions. These observations have been confirmed by others (3), (4), (5). It has also been shown (6) that the cerebral cortex, cerebellum, midbrain and medulla of the rabbit's brain, after removal from the skull, are affected differently in their water absorption by such substances as glucose, NaCl, HCl and creatinine. To determine the effect produced on the water content of the brain as a whole or of the different parts of the brain *in vivo*, by various chemicals introduced into the blood stream, is extremely difficult. If definite weight relations were established the problem would be simplified.

This, however, as far as we could ascertain, heretofore has not been done. Cuvier (7), over a hundred years ago, observed that differences in the relationships between the weight of the brain and the body weight were so great in different animals of the same species that he thought it impossible to establish a comparative relationship. One worker, he says, gives the ratio for the cat 1 to 305, another 1 to 47. In the century and more that has elapsed since Cuvier a number of studies have confirmed his observations. Ellenberger and Baum (8) find that a wide variation occurs in the brain weight among animals of the same species. Their figures for the horse are 372 to 570 grams, for the cow 410 to 530 grams, for the pig 96 to 145 grams, and for the dog 66 to 138 grams. A similar variation also exists in the ratio of the weight of the brain to the body weight (brain quotient). The brain quotient of the horse varies from 1:418 to 1:1000; of the dog 1:30 to 1:400; of the pig 1:1200 to 1:1900.

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Brown and his co-workers (9) found that although "there is a common tendency on the part of all the organs to weigh more or less according to the weight of the animal," nevertheless a constant relation is not maintained between organ weight and body weight. The weight of the brain in particular (and also the weight of other organs such as the hypophysis and the kidney) appears to be more or less independent of the body weight (10). These conclusions are drawn from data obtained from the body weight and organ weight of three hundred and fifty rabbits. Continuation of this investigation to the extent of six hundred and forty-five animals confirmed, in general, these observations (11), (12).

TABLE 1

	CEREBRAL HEMISPHERES (NORMAL RABBIT)				CEREBELLUM (NORMAL RABBIT)		
	Wet weight of cerebral hemispheres to total wet weight of brain	Dry weight of cerebral hemispheres to total dry weight of brain	Dry weight of cerebral hemispheres to wet weight of cerebral hemispheres	Total dry weight of brain to total wet weight of brain	Wet weight of cerebellum to total wet weight of brain	Dry weight of cerebellum to total dry weight of brain	Dry weight of cerebellum to wet weight of cerebellum
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Age, 6 months							
Number of animals, 7							
Average	58.5	54.5	20.3	21.9	15.2	15.0	21.7
Average deviation	0.6	0.9	0.5	0.6	0.6	0.6	0.6
Age, 12 months							
Number of animals, 14							
Average	59.6	55.2	20.3	22.0	14.1	14.1	21.8
Average deviation	0.7	0.8	0.7	0.6	0.8	0.7	0.6
Age, 18 months							
Number of animals, 8							
Average	59.1	54.9	20.4	22.0	14.7	14.7	21.9
Average deviation	0.7	0.7	0.4	0.5	1.0	0.8	0.9

It was therefore evident to us that we could not use the ratio of body weight to organ weight as a norm for measuring changes in the water content of the brain. Two possibilities were suggested: 1, if the percentage water content of the entire brain or various portions of the brain is constant, or 2, if the ratio between the weight of the brain and the weight of the skull is constant, these relationships would serve our purpose.

PROCEDURE. Rabbits of a known age were decapitated and the brain quickly removed from the skull. The brain was laid on a glass plate and divided into four portions,—cerebral hemispheres, cerebellum, midbrain and medulla. As this procedure required only a few minutes we did not deem it necessary to work under a glass hood. Each portion of the brain

was placed in a weighing bottle and weighed immediately. The bottles with stoppers removed were then placed in a drying oven at constant temperature. Each bottle was weighed on successive days until the weight remained constant. The weight of the contents was determined by subtracting the weight of the bottle (dried) from the total weight of the bottle

TABLE 2

	MIDBRAIN (NORMAL RABBIT)			MEDULLA (NORMAL RABBIT)		
	Wet weight of midbrain to total wet weight of brain	Dry weight of midbrain to total dry weight of brain	Dry weight of midbrain to wet weight of midbrain	Wet weight of medulla to total wet weight of brain	Dry weight of medulla to total dry weight of brain	Dry weight of medulla to wet weight of medulla
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Age, 6 months						
Number of animals, 7						
Average	16.1	17.7	24.1	10.1	12.7	27.4
Average deviation	0.3	0.4	0.6	0.4	0.6	0.7
Age, 12 months						
Number of animals, 14						
Average	15.9	17.4	24.1	9.9	12.6	27.5
Average deviation	0.9	1.1	0.8	0.5	0.7	0.6
Age, 18 months						
Number of animals, 8						
Average	16.3	18.1	24.5	9.8	12.4	27.8
Average deviation	0.4	0.4	0.6	0.3	0.5	0.7

TABLE 3

TOTAL WET WEIGHT OF BRAIN TO WEIGHT OF SKULL	TOTAL DRY WEIGHT OF BRAIN TO WEIGHT OF SKULL
per cent	*per cent*
42.3	7.9
35.9	7.6
44.3	10.3
39.7	8.5
47.4	10.1

and its contents. The percentages recorded in the tables were calculated from the results.

All parts of the skull were carefully collected after the brain had been removed, cleaned by boiling with strong soap powder, dried and weighed.

The age of the animals was within two or three weeks of the ages given in the tables. They came from different litters and varied in body weight within wide limits. No effort was made to regulate the diet.

RESULTS. It soon became apparent, in the course of our observations,

that the percentage dry weight of the wet weight in the entire brain and in the four parts,—cerebral hemispheres, cerebellum, midbrain and medulla —is constant. The average deviations are approximately 0.5 per cent. This constancy of the percentage values, it should be observed, was obtained from animals taken at random from different litters and without controlling, in any way, the diet. Had the dissections been made under a glass hood, it is possible that the deviations would have been reduced.

The highest percentage of water is contained in the cerebral hemispheres. The other parts follow in the order: cerebellum, midbrain, medulla. Calculated on the basis of 100 parts of water, the dry substance of the four portions of the brain in animals 12 months old is as follows: cerebral hemispheres, 20.3; cerebellum, 21.8; midbrain, 24.1; medulla, 27.5. It follows from these data that each portion of the brain has a characteristic normal capacity for water absorption.

As the age advances from 6 months to 18 months there is only a very slight difference in the percentage water content of the entire brain and the four parts of the brain. But, since the increase is so constant throughout the experiment, we conclude that in animals 18 months old there is relatively less water in all parts of the brain, although the difference is very small.

It is interesting to observe that the ratio of the wet weight of the cerebellum to the total wet weight of the brain is always the same as the ratio of the dry weight of the cerebellum to the total dry weight of the brain. We are unable, at present, to interpret this relationship as having any significance.

The differences in the percentage wet or dry weight of the brain to the weight of the skull are so wide in different animals that this ratio has no value in determining volume changes in the brain.

SUMMARY

The ratio of the dry weight of the entire brain to the wet weight is constant.

The same constancy of relationship is found in the cerebral hemispheres, cerebellum, midbrain and medulla.

There occurs only a very small difference in the ratio of the dry weight of the entire brain and the four parts to the respective wet weights in animals 6 months, 12 months and 18 months old. The difference however is so consistent that it is probably significant.

There is a pronounced and constant difference in the ratio of the dry weight to the wet weight in the cerebral hemispheres, cerebellum, midbrain and medulla.

The cerebral hemispheres contain the highest percentage of water.

The percentage content of the other parts follow in the order,—cerebellum, midbrain, medulla.

For each 100 parts of water in rabbits twelve months old the weight of the dry substance is as follows: cerebral hemispheres, 20.3; cerebellum, 21.8; midbrain, 24.1; medulla, 27.5.

The difference in ratio among the various parts of the brain shows that the water absorption capacity is different for the four parts of the brain.

The constancy in ratio for a given portion of brain substance indicates that the various portions of the brain have each a characteristic normal water absorption capacity.

The ratio of the wet and dry weight of the brain to the weight of the skull is not constant.

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EFFECTS PRODUCED ON WEIGHT RELATIONS IN THE RABBIT'S BRAIN BY ANESTHETICS AND INTRAVENOUS INJECTIONS

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The experiments reported in this paper were undertaken to determine the effect produced on the water content of different parts of the brain by chemical changes induced in the blood stream. The constancy that obtains in the ratio of the dry weight to the wet weight of various portions of the normal rabbit's brain (1) afforded a means of carrying out the present investigation.

METHOD. Rabbits twelve months old (within two or three weeks) were used throughout these experiments. They were injected subcutaneously and intravenously with $MgSO_4$ and urea in solution, subcutaneously with morphine sulfate and anesthetized with ether and chloroform. Immediately after decapitation the brain was divided into cerebral hemispheres, cerebellum, midbrain and medulla. The weights were determined in the manner described in a previous report (1). Since an absolutely uniform procedure was not followed for the various injections the following protocols are given.

Rabbits 30, 31, 32 and 33 received subcutaneously for four consecutive days 10 cc. $MgSO_4$. Decapitated one hour after last injection.

Rabbit 34 received subcutaneously for three consecutive days 15 cc. 25 per cent $MgSO_4$. The fourth day 5 cc. 5 per cent solution intravenously. One hour later the same amount. Decapitated three hours after the last injection.

Rabbit 35 received 3 cc. 10 per cent $MgSO_4$ intravenously. One hour and forty-five minutes later 2.5 cc. Decapitated ten minutes after last injection.

Rabbit 36 received 5 cc. $MgSO_4$ intravenously. Same amount one hour later. Decapitated one hour after last injection.

Rabbit 37 received 2 grams urea in solution subcutaneously for four consecutive days. Decapitated two hours after last injection.

Rabbit 38 received 2 grams urea in solution subcutaneously for three successive days. One gram dissolved in NaCl injected intravenously on the fourth day. Same amount injected one hour later. Decapitated one hour after last injection.

Rabbit 39 received 2 grams urea in solution subcutaneously for four successive days. The same amount injected on the fourth day. Decapitated one hour later.

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Rabbits 40–44 were kept covered by a bell jar under light ether anesthesia approximately three hours. They were then removed from the bell jar, placed under heavy anesthesia for about ten or fifteen minutes and decapitated.

TABLE 1

	CEREBRAL HEMISPHERES			CEREBELLUM	
	Wet weight of cerebral hemispheres to total wet weight of brain	Dry weight of cerebral hemispheres to wet weight of cerebral hemispheres	Total dry weight of brain to total wet weight of brain	Wet weight of cerebellum to total wet weight of brain	Dry weight of cerebellum to wet weight of cerebellum
Rabbit injected with $MgSO_4$					
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Number of animals, 7 (30–36)					
Average	59.4	20.8	22.4	14.2	21.7
Average deviation	0.9	0.4	0.5	0.6	0.3
Rabbit injected with urea solution					
Number of animals, 3 (37–39)					
Average	58.4	20.6	21.8	13.9	21.5
Average deviation	0.7	0.4	0.4	0.5	0.5
Rabbit anesthetized with ether					
Number of animals, 5 (40–44)					
Average	58.2	19.6	21.5	15.2	21.1
Average deviation	0.7	0.4	0.6	0.9	0.9
Rabbit anesthetized with chloroform					
Number of animals, 3 (45–47)					
Average	56.3	20.3	21.2	13.8	21.7
Average deviation	1.3	0.3	0.7	1.0	0.3
Rabbit injected with morphine					
Number of animals, 4 (48–51)					
Average	57.6	20.0	21.3	15.6	20.9
Average deviation	0.7	0.4	0.3	0.5	0.4

Rabbits 45–47 were placed under chloroform anesthesia in the same manner as rabbits 40–44.

Rabbit 48 received subcutaneously 0.25 grain morphine sulfate. Two hours later 0.5 grain. Animal in heavy stupor. Decapitated two and one-half hours later.

Rabbit 49 received 0.25 grain morphine. One and one-half hours later 0.25 grain. Animal in stupor. Decapitated one hour and twenty minutes after last injection.

Rabbit 51 received 0.25 grain morphine. One hour and fifteen minutes later 0.25 grain. Animal in stupor. Decapitated two hours and forty-five minutes after last injection.

TABLE 2

	MIDBRAIN		MEDULLA	
	Wet weight of midbrain to total wet weight of brain	Dry weight of midbrain to wet weight of midbrain	Wet weight of medulla to total wet weight of brain	Dry weight of medulla to wet weight of medulla
Rabbit injected with $MgSO_4$				
	per cent	*per cent*	*per cent*	*per cent*
Number of animals, 7 (30–36)				
Average	16.1	24.6	10.4	28.0
Average deviation	0.7	0.4	0.7	0.5
Rabbit injected with urea solution				
Number of animals, 3 (37–39)				
Average	17.1	24.9	10.6	27.9
Average deviation	1.1	0.6	0.6	0.7
Rabbit anesthetized with ether				
Number of animals, 5 (40–44)				
Average	16.0	23.6	10.7	26.7
Average deviation	0.6	0.6	0.4	0.6
Rabbit anesthetized with chloroform				
Number of animals, 3 (45–47)				
Average	17.2	24.1	11.0	27.1
Average deviation	1.0	0.3	0.1	0.2
Rabbit injected with morphine				
Number of animals, 4 (48–51)				
Average	16.5	23.8	10.4	27.1
Average deviation	0.2	0.7	0.2	0.7

RESULTS AND DISCUSSION. The data of these experiments show, by comparison with those of normal animals (cf. 1) that the percentage water content of the entire brain and the four parts of the brain is affected by

various chemical changes in the blood stream. Examining the figures presented in the tables, one sees that a specific chemical compound may produce different effects on the hydration of the four parts of the brain,—cerebral hemispheres, cerebellum, midbrain and medulla.

In a previous paper we expressed the view that the physiological basis of mental disorders might possibly be found in physico-chemical conditions of the central nervous system, or in a disturbance of the dynamic equilibrium existing between the brain protoplasm and the blood. Reviewing the literature on the subject and observing the current therapeutic practices, one is struck by the paucity of our knowledge of the physiological conditions underlying mental disorders. Extensive analyses of the blood of the insane have led to no practical results. Nevertheless, when one considers the intimate relationship that exists between the brain and mental life, as proved by the effect of drugs, etc., on mental processes, he has every reason to believe that there is a physiological basis for the protean pathological conditions grouped under the convenient term, insanity. We may assume, therefore, that the integrity of mental life depends upon the "normal" physiological functioning of the brain protoplasm. According to this conception, any condition of the blood (or of the brain matter itself) which affects the physiological processes of the brain, would have a corresponding effect on mental processes. We do not intend to minimize the complexity of the system or of the mechanisms involved, but we suggest that physico-chemical changes in the brain protoplasm such as increased or decreased hydration, should be considered as a possible important factor. These physico-chemical changes might have both a direct effect on mental processes and an indirect effect by modifying the physiological processes in the brain substance.

These experiments do not give an answer to the many questions that are implicitly contained in the foregoing discussion. They prove that certain chemical changes in the blood affect the hydration of the brain, and that the effect produced by a given substance is not necessarily the same for the different parts of the brain. Whether some similar physico-chemical alteration occurs in the brain protoplasm of the insane because of toxins in the blood, secretions of the endocrines or the products of disordered metabolism can be determined only by further research. The present study merely suggests such a possibility.

SUMMARY

From the data presented it is concluded that chemical changes in the blood stream affect the water content of the brain.

A chemical compound such as $MgSO_4$ may produce different effects on

the hydration of the four parts of the brain,—cerebral hemispheres, cerebellum, midbrain and medulla.

The water content of the entire brain is reduced by the intravenous injection of $MgSO_4$ and also, to a slight extent, by the intravenous and subcutaneous injection of urea. It is increased by ether, chloroform and morphine anesthesia.

The water content of the four parts of the brain is affected as follows:

Cerebral hemispheres: Reduced by $MgSO_4$ and urea. Increased by ether and morphine. Not affected by chloroform.

Cerebellum: Not affected by $MgSO_4$ or chloroform. Increased by urea, ether and morphine.

Midbrain: Reduced by $MgSO_4$ and urea. Increased by ether and morphine. Not affected by chloroform.

Medulla. Reduced by $MgSO_4$ and urea. Increased by ether, morphine and chloroform.

No attempt has been made to determine whether these are direct or indirect effects.

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EFFECT ON RESPIRATION, BLOOD PRESSURE AND CAROTID PULSE OF VARIOUS INHALED AND INSUFFLATED VAPORS WHEN STIMULATING ONE CRANIAL NERVE AND VARIOUS COMBINATIONS OF CRANIAL NERVES

III. Olfactory and Trigeminals Stimulated

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Two problems are undertaken in this report. 1. To determine whether the respiratory and circulatory changes following short inhalations of various vapors in *olfactory* rabbits (trigeminal cut) and in *trigeminal* rabbits (olfactory fibers cut) are to be attributed chiefly to direct stimulation of the olfactory and trigeminal nerves or to—a, humoral-peripheral and humoral-medulla stimulation from absorption of these vapors; b, secondary humoral stimulation due to the cessation of respiration; c, secondary mechanical-vagus stimulation or lack of stimulation due to cessation of respiration. 2. A comparison is made in olfactory and trigeminal rabbits of the respiratory and circulatory changes obtained from inhalations of a large number of odoriferous and irritating vapors.

Literature. Kratschmer, Gourewitsch, Henry and Verdin, Sandmann, Beyer, Seemann, Roger, and Mayer, Magne and Plantefol state that projection or inhalation of an irritant into the nasal cavities causes a marked inhibition or cessation of respiration.

Mayer,. Magne and Plantefol (8), working chiefly with halogens and chloro-methyl-carbonates, concluded that in the upper respiratory tract the response to irritants was greater than for odors, and that dogs were less sensitive to these vapors than rabbits.

According to Kratschmer (1) ammonia, carbonic acid, chloroform, tobacco smoke, electrical and mechanical stimulation of the nasal mucosa do not elicit a respiratory response after sectioning the trigeminal roots.

Beyer (5) maintains that the effects of ammonia and acetic acid are stronger on respiration after sectioning the olfactory than after division of the trigeminal. Agreeable odors are said to accelerate respiration and disagreeable to evoke no change. According to Beyer the coal tar products, especially xylol, cause a complete arrest of respiration. This reaction is said to be fully or even more marked with the trigeminal severed. The aromatic and etheral odors are conducted chiefly, if not entirely, by the olfactory nerve.

Kratschmer (1), Beyer (5), Roger (7), and Brodie and Russell (9) obtained circulatory changes from the projection or inhalation of irritants in the nostrils. Kratschmer and Roger always obtained a preliminary drop followed by a rise in blood pressure, but no preliminary drop is shown in their tracing when the vagi are sectioned. Beyer's peppermint tracing also shows a preliminary drop followed by a rise.

Comparison between the action of certain vapors on respiration and circulation in tracheotomized and in non-tracheotomized rabbits. In this experiment it is apparent that the possibility of humoral-peripheral and humoral-medulla stimulation as the result of absorption is readily eliminated by having the animal obtain its ventilation from a tracheal cannula and insufflating the stimulating vapor into the nostrils, using the same general technical procedure described in the first paper of this series. Olfactory rabbits were used and in nearly every case they were obtained by bilateral severance of the naso-ciliary and maxillary nerves, which was shown in the first report to be as effective as severance of the trigeminal root intracranially. Autopsy was always made to ascertain whether these nerves had been sectioned.

A control graph (fig. 1 A) from olfactory rabbit 616 reveals no change in respiration or circulation from insufflating air saturated with water into the nostrils.

The first graph from xylol insufflation (fig. 1 B) obtained from rabbit (616) exhibits a complete suppression and subsequent depression of respiration, a 22 mm. rise in blood pressure, a slowing of the carotid pulse of 3 beats in two seconds, and an increase of 1 mm. in the height of the pulse waves. It required forty seconds after insufflation for respiration and circulation to regain their normal. Two other xylol records demonstrate slightly weaker respiratory and circulatory changes. An ammonia graph discloses respiration greatly suppressed and brought to a standstill, a rise of 19 mm. in blood pressure, a slowing of the pulse 1.5 in two seconds, and a 1 mm. increase in height of the pulse waves during insufflation.

The xylol and ammonia insufflations were repeated in three other olfactory rabbits with like results.

In a fifth olfactory rabbit (no. 621) this experiment was carried one step further by severing the depressors, vagi and cervical sympathetics at the level of the larynx. This should eliminate any secondary humoral or mechanical-peripheral vagus stimulation or lack of stimulation due to cessation of respiration. Under these conditions insufflations of xylol into both nostrils resulted in a cessation of respiration, a rise in blood pressure of 21 mm., a pulse reduction of 1+ wave in two seconds, but without change in the height of the pulse waves.

One benzol record from the above rabbit portrays a decided drop in blood pressure with no subsequent rise.

For comparison with the xylol insufflation record 1 B, the xylol inhala-

tion record 1 C is selected. It is representative of some twenty graphs taken from a number of olfactory rabbits under veronal-sodium in which the vagi and depressors were sectioned. During stimulation figure 1 C reveals respiration at a standstill, a 12 mm. rise in blood pressure, a

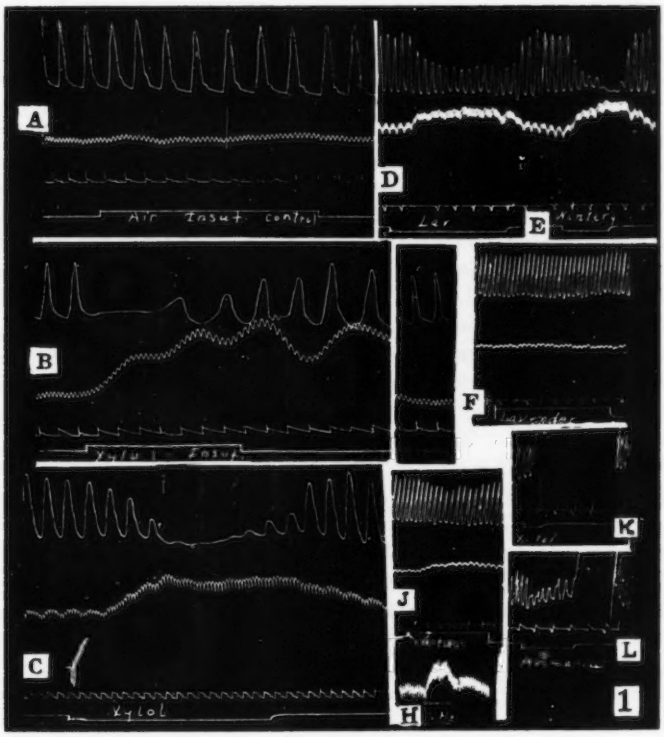

Fig. 1 A and B. Thoracic respiratory and blood pressure tracings, insufflations into the nostrils of a tracheotomized olfactory rabbit, veronal-sodium, time in seconds, space in B represents a one minute interval; C, inhalation-thoracic respiratory and blood pressure tracings, olfactory rabbit, depressors and vagi cut, veronal-sodium, time in seconds; D, E and H, same as C, time in five seconds; H, blood pressure only; K and L, inhalation-thoracic respiratory tracings from olfactory rabbit, no hypnotic, time in five seconds; F and J, inhalation-thoracic respiratory and blood pressure tracings from trigeminal rabbit, veronal-sodium, time in five seconds.

decrease in the rate of the pulse of 1+ in two seconds, and an increase of 2.5 mm. in the height of the pulse waves. Tracings taken during the inhalation of ammonia and acetic acid closely resemble figure 1 C.

Numerous inhalation tracings described in the next section of this paper

from trigeminal rabbits, both before and after double vagotomy, are very similar to the insufflation records described in the first paper of this series, where the animals were tracheotomized and the maxillary branches of the trigeminus were the only functioning, intact, afferent nerves to the nasal mucosa.

COMMENT. It is apparent from a comparison of the insufflation-tracheotomy-vagotomy, respiratory and blood pressure graphs in both olfactory and trigeminal rabbits with similar inhalation-vagotomy graphs that the two are very similar, and that the factors enumerated in the first paragraph of this report take but little part in the general inhalation reactions. The fact that the changes in respiration and circulation are a little more pronounced in the insufflation experiments is probably to be attributed to a higher concentration of the vapors. The writer hopes in the future to be able to explain the cause of the changes in the pulse which take place in figure 1 C and other inhalation graphs after the depressors and vagi have been sectioned.

INHALATION EXPERIMENTS. *Effect of various inhalants on respiration and circulation in normal rabbits.* Thoracic-respiratory and carotid blood pressure tracings were taken from rabbits given two-thirds of a full anesthesia dose of veronal-sodium intravenously and from others not under a hypnotic. The latter were strapped to a board, blindfolded and their ears were plugged with cotton. The respiration in these animals was at first very irregular and greatly accelerated, but finally it became normal. In general the first few graphs were worthless on account of body movements, but after a time fairly reliable tracings could be obtained with only an occasional irregularity. The inhalants were administered from cones held above the nostrils, the distance varying roughly in inverse ratio to the strength of the vapors. At first, for control, a cone saturated with water or some volatile non-odorous substance was placed above the nostrils immediately preceding the cone containing the inhalant, but inasmuch as the first cone never altered respiration or circulation this procedure was discontinued.

Tabulated data compiled from the respiratory and the blood pressure tracings from two normal rabbits under veronal-sodium and two without any hypnotic permit of the following summary. The agreeable odors, oil of bergamot, lavender and anise always show some depression and inhibition of respiration during the period of inhalation. Oil of cloves, orange and rose tracings from one rabbit under veronal-sodium and both non-hypnotized rabbits reveal some depression or depression and inhibition of respiration, least marked in the oil of cloves tracings. Of the disagreeable odors inhaled, none of the asafetida records exhibit any respiratory changes, and only one butyric acid tracing from one of the non-hypnotized rabbits demonstrates any depressor or inhibitory changes in respiration.

The tracings obtained from such substances as peppermint, alcohol, menthol, eucalyptus and camphor always show considerable depression and inhibition of respiration. Respiration is at a complete standstill in all of the wintergreen, ether, chloroform, pyridin and phenol tracings. Records obtained from these inhalants in the non-hypnotized rabbits disclose a sneeze in addition to the complete arrest of respiration. Practically all of the xylol, benzol, formalin, oil of mustard, acetic acid and ammonia graphs from both the veronal-sodium and the undepressed rabbits reveal both a complete suppression of respiration and the sneeze. In the undepressed rabbits the sneeze appears very quickly after inhalation of ammonia, and in both the ammonia and xylol tracings it occurred when the cones were held 8 cm. from the nostrils.

All of the blood pressure tracings which correspond to strong and moderately strong respiratory changes disclose a rise in blood pressure, a slowing and strengthening of the pulse which are in direct proportion to the alteration in respiration. There is never an inhalation-respiratory change without a corresponding circulatory change.

The following tabulated vagus and vagotomy respiratory and circulatory data obtained from a normal rabbit (no. 581) under veronal-sodium at the time of inhalation of ammonia is very similar to corresponding data obtained a second normal rabbit under veronal-sodium.

	BEFORE STIMULATION	DURING STIMULATION
All nerves intact (1)		
Rate of carotid pulse waves	8.5 in 2 sec.	6 in 2 sec
Height of carotid pulse waves	1.5 mm.	3 mm.
Blood pressure rise		16 mm.
Respiration		Standstill
After cutting depressors (2)		
Rate of carotid pulse waves	10 in 2 sec.	6.5 in 2 sec.
Height of carotid pulse waves	0.5 mm.	3 mm.
Blood pressure rise		20 mm.
Respiration		Standstill
After cutting vagi (3)		
Rate of carotid pulse waves	8 in 2 sec.	7 in 2 sec.
Height of carotid pulse waves	1.5 mm.	2.5 mm.
Blood pressure rise		15 mm.
Respiration		Standstill
After cutting cer. sym. (4)		
Rate of carotid pulse waves	6.5 in 2 sec.	6 in 2 sec.
Height of carotid pulse waves	1.5 mm.	2 mm.
Blood pressure rise		11 mm.
Respiration		Standstill

It is apparent from this table that the relative strength of the circulatory reactions is in the order (2), (1), (3), (4), and the effect on respiration is the same in all four tracings.

A benzol-inhalation graph from a normal cat (no. 580) under veronal-sodium shows a marked depression of the respiratory excursions, a 30 mm. rise in blood pressure, some increase in the height of the pulse waves, but no change in their rate.

Respiratory tracings from a normal rabbit (no. 533) under a full dose of urethan-chloretone portray very similar changes to the rabbits under veronal-sodium for the strong irritating inhalants, but for the mild irritants, the depression and inhibition is only moderate, and there is no response to the agreeable or disagreeable odors, even the disgusting odor of a skunk elicited no response.

The xylol and benzol respiratory tracings from a normal cat (no. 529) under urethan-chloretone reveal but little depression and inhibition.

In some experiments to be reported in a later paper on a pseudo-vagal reaction, a large number of blood pressure graphs were taken from the right vena cava close to the atrium, at the time a rabbit under veronal-sodium was inhaling various substances. A long cannula was inserted into the right jugular until it reached the right superior vena cava, the subclavians and azygos being then about the only veins of any consequence left to discharge their blood into the right superior vena cava. The pressure was recorded by a small saline manometer especially made for this purpose. At the same time the carotid pressure and its pulse were recorded by a mercury manometer.

A venous tracing taken at the time of inhaling benzol reveals a rise in pressure of 8+ mm. as compared with an 18 mm. rise for the carotid. The superior vena cava rise takes place fully as early as the carotid, is more gradual, and the return to normal is quicker. The venous pulse (atrial) waves disclose the same amount of slowing as the carotid waves, namely, a drop of one beat in two seconds. Instead of showing an increase in height of the pulse waves, the venous pulse waves portray a marked decrease in height.

Effect of various inhalants on respiration and circulation in olfactory rabbits. Attention is directed at the outset to the results of the introductory experiments of this report and to the results of the first paper of this series, which indicate that the alteration of respiration and circulation following short inhalations of any vapor, when the naso-ciliary and maxillary nerves are divided on both sides, takes place primarily through stimulation of the olfactory nerve endings. Of six *olfactory* rabbits used in this experiment, two were prepared by sectioning the trigeminal roots intracranially[1]

[1] In rabbits where both trigeminal roots are to be divided intracranially, it is advisable to sever them on different days. A special instrument for this purpose was made from a piece of 2 mm. steel wire. After flattening one end of the wire and bend-

and four by cutting the naso-ciliary and maxillary nerves on both sides. The first two, designated as 1 and 2, had no hypnotic, while the latter, 3 to 6, were given two-thirds to a full dose of veronal-sodium intravenously. In 5 and 6, the depressors and vagi were sectioned and simultaneous thoracic-respiratory and blood pressure tracings were recorded. In 3, respiratory records were obtained before and during hypnosis.

The following generalizations can be made from tabulated data compiled from many inhalation tracings taken from these six olfactory rabbits. The agreeable odors, oil of cloves, rose, orange, lavender, and bergamot cause some depression or depression and inhibition of respiration in rabbits 1, 3, 4, and 5. In rabbit 2 there is some acceleration and depression of the respiratory excursions during the first part of the inhalation period of the agreeable odors and the disagreeable odor, butyric acid, but during the middle and last part of the inhalation period the tracings show an inhibition in place of acceleration; so that the total effect of inhalation of these odors in rabbit 2 would have to be regarded as one of reduced ventilation. Inhalation of rose, orange and oil of cloves elicited no change in respiration or circulation in rabbit 6, but oil of bergamot and lavender (fig. 1 D) altered both respiration and circulation.

Practically all of the oil of anise, peppermint, camphor, menthol, eucalyptus, and alcohol graphs from all six rabbits show considerable depression and inhibition of respiration, together with a rise in blood pressure and some pulse changes.

All of the respiratory tracings obtained during the inhalation of oil of wintergreen (fig. 1 E), ether, chloroform, phenol, pyridin, oil of mustard, formalin, acetic acid, ammonia, benzol, and xylol (fig. 1, C) disclose either a pronounced depression and inhibition or a complete arrest of respiration. Practically all of the respiratory records from the above vapors also elicited a sneeze in the non-hypnotized rabbits 1 and 2, as shown by the xylol and ammonia tracings (fig. 1, K and L).

In olfactory rabbits 5 and 6 the changes in blood pressure and pulse are in direct relationship to the strength of the inhalant affecting respiration, as is revealed by the lavender, wintergreen, and xylol tracings (fig. 1, D, E and C).

Attention is directed to the strength of the respiratory and circulatory

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ing it to fit the contour of the sides and base of the skull, the tip end of the convex surface was ground away on either side so as to leave a median blade which was fairly sharp. With the animal under ether, the operative procedure consisted of cutting a hole about a centimeter in diameter through the temporal bone in front of the external auditory meatus. The flattened end of the instrument was next inserted through the temporal orifice until it reached the place where the trigeminal root leaves the Gasserian ganglion; a few backward and forward movements were sufficient to sever a root with little injury to the brain and none to the olfactory area.

reactions obtained in all the olfactory rabbits from inhalations of the strongly irritating vapors, acetic acid, oil of mustard, ammonia (fig. 1 H, blood pressure only), etc. They will probably have to be ranked next to xylol and benzol as olfactory stimuli.

In two olfactory rabbits not included in the six described previously, where urethan-chloretone was used for hypnosis, the threshold for stimulation was much higher than it was for veronal-sodium. The xylol and benzol respiratory tracings from these rabbits show only a moderate depression and inhibition. Furthermore, the threshold of stimulation of the olfactory nerves by inhalants is demonstrated to be much higher in cats than in rabbits.

Effect of various inhalants on respiration and circulation in trigeminal rabbits. It is apparent from the first paper of this series and the preliminary experiment of this report, that short inhalations of various vapors which alter respiration and circulation in trigeminal rabbits, do so chiefly through stimulation of the sensory endings of the naso-ciliary and the maxillary nerves.

Trigeminal rabbits were prepared by sectioning the olfactory fibers after the manner described in the first paper of this series. Rabbit 1 had no hypnotic and only thoracic-respiratory tracings were obtained. Respiratory tracings were taken from rabbit 2 both before and after giving veronal-sodium. Rabbits 3 and 4 had a two-thirds dose of veronal-sodium and simultaneous respiratory and circulatory records were taken during the time various odoriferous and irritating vapors were inhaled from cones held above the nostrils. Before administering inhalants to trigeminal rabbits not under a hypnotic, the animal was blindfolded, its ears were plugged with cotton, and all of the sensory hairs about the snout were clipped.

A mass of tabulated data taken from these graphs may be presented in the following manner. There is no alteration of respiration or circulation in rabbits 1 to 4 at the time of inhalation from cones saturated with water, hydrogen peroxide and 50 per cent alcohol. Negative results are also obtained in all four rabbits for the agreeable inhalants, oil of cloves, rose, orange, lavender (fig. 1 F), and for the disagreeable odors asafetida, fresh cat's urine, and butyric acid.

Oil of bergamot, wintergreen (fig. 1 J), and anise always elicit some depression and inhibition of respiration and some rise in blood pressure. Menthol, eucalyptus, and 95 per cent alcohol produce about the same changes in respiration and circulation as shown in the wintergreen tracing. In rabbit 2 the respiratory reactions are somewhat more pronounced before veronal-sodium than afterward. Before veronal-sodium, respiration comes practically to a standstill in some of the anise and bergamot tracings, and in the 95 per cent alcohol tracing the suppression of respira-

tion is followed by sneezing. There was a marked tendency in rabbit 1 to sneeze the instant an inhalant was perceived. The only way that a normal tracing could be obtained was to bring the cones very slowly to the nostrils and often this procedure would fail.

The strongly irritating vapors, ether, chloroform, pyridin, phenol, formalin, acetic acid, and ammonia evoke in all four rabbits a marked depression and inhibition of respiration, amounting in most instances to a complete suppression. There is also a considerable rise in blood pressure and a slowing and strengthening of the pulse. In all of the graphs obtained

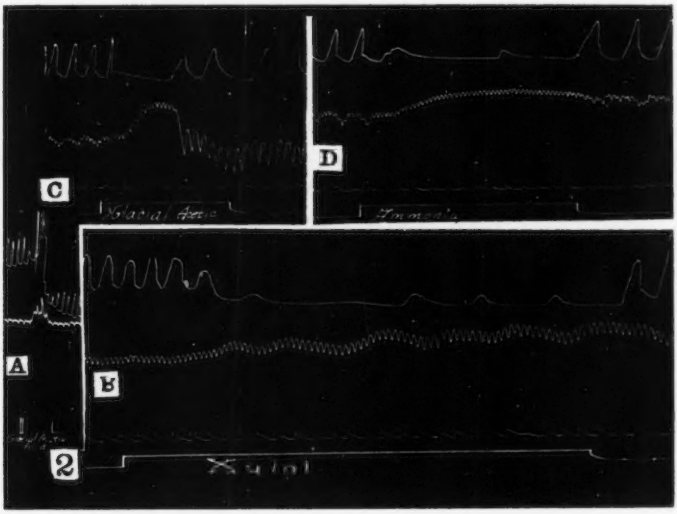

Fig. 2 A. Same as figure 1 F and J; B, inhalation-thoracic respiratory and blood pressure graphs from trigeminal rabbit, veronal-sodium, time in seconds; C, same as B except depressors cut; D, same as C except vagi cut.

from the undepressed rabbits 1 and 2 and all of the acetic acid (fig. 2 A), and ammonia graphs from the veronal-sodium rabbits 3 and 4 the arrest of respiration is followed by a sneeze. It should be noted that the cones containing the milder irritants were held close to the nostrils.

The records from the coal tar products, xylol and benzol, are of especial interest in view of the fact that Beyer regarded xylol as a specific stimulus affecting respiration over the olfactory nerves. All of the xylol and benzol tracings from the undepressed rabbits 1 and 2 and a large number from the veronal-sodium rabbits 3 and 4 disclose a sneeze in addition to the suppression of respiration. Figure 2 B, which is a xylol graph from a

fifth trigeminal rabbit (587) under a full dose of veronal-sodium, reveals respiration practically at a standstill, a rise in blood pressure of 10 mm., a decrease of 1.5 pulse waves in two seconds, and an increase in the height of the pulse waves of 1.5 mm.

A number of records from trigeminal rabbit 555 under urethan-chloretone do not show any respiratory or circulatory changes as a result of inhaling oil of bergamot, anise, peppermint, and wintergreen. Alcohol, ether, chloroform, benzol, xylol, acetic acid, and ammonia evoke considerably weaker respiratory and circulatory responses than the corresponding veronal-sodium records exhibit.

Figure 2 C illustrates a *pseudo-vagal reaction* occurring in an acetic acid-inhalation tracing from a trigeminal rabbit. It consists of a drop in blood pressure, an immediate strengthening and slowing of the pulse, the latter being exactly one-half the previous rate. These gigantic pulse waves continue for a considerably longer time after inhalation ceases than is the rule for pulse changes following nerve stimulation. This reaction may appear at any time during stimulation, after stimulation ceased, or it may take place spontaneously in vagotomized rabbits. Nerve fatigue and benzol stimulation appear to bring it on. This reaction which will be described in detail in a subsequent paper, is probably an ectopic rhythm. It may be present in some of the tracings of other investigators.

Figure 2 D is an ammonia graph from the same trigeminal rabbit as B and C, but obtained after the depressors and vagi were sectioned. It is obvious that respiration is practically at a standstill. That there is no sneeze is due to the fact that the cone was purposely held at considerable distance from the snout. There is a 7 mm. rise in blood pressure, a decrease in the rate of the pulse of one beat in four seconds, and no change in the height of the pulse waves.

It is seen from comparing this record and others like it with similar ammonia graphs taken before vagotomy together with several xylol graphs from this and other trigeminal rabbits with figure 2 B, that sectioning the depressors and vagi does not materially affect the respiratory changes obtained from inhalations. There is, however, less rise in blood pressure and the pulse changes are considerably reduced after sectioning these nerves.

Comments. In rabbits the olfactory and trigeminal nerves protect the lungs from dangerous and irritating vapors by suppression of respiration and by the production of a sneeze.

The chief result of olfactory stimulation from inhalants is one of suppression of respiration; a sneeze is evoked only in non-anesthetized rabbits. Trigeminal stimulation causes both a suppression of respiration and a sneeze; the sneeze is absent in weak stimulation and comes on early in

strong stimulation. As shown in the previous report the vagus reaction is concerned primarily with a cough or spasm, the suppressor effect while usually present, is of little significance except in very weak stimulations.

Contrary to Beyer (5) the general effect from inhalations of agreeable odors is one of depression and inhibition of respiration. Some agreeable odors are effective over the trigeminal nerve as well as the olfactory. In agreement with Beyer my records show that disagreeable odors rarely ever stimulate the olfactory or trigeminal nerves. Also the coal tar derivative xylol proved to be the most effective olfactory stimulant. In addition it is a very efficient trigeminal stimulant. On the other hand, the strong irritants usually classified as trigeminal stimulants are very effective olfactory stimulants also.

Obtaining negative results from cones containing 50 per cent alcohol and from cones containing the non-odorous and non-irritating hydrogen peroxide, demonstrates that the small alcohol content in some inhalants and the evaporation of a volatile substance are not factors for stimulation in any of the foregoing experiments.

Urethan-chloretone apparently raises the threshold of stimulation from inhalations much more than veronal-sodium. The sneeze is more easily elicited in rabbits not under a depressant or under two-thirds of a full anesthesia dose of veronal-sodium than it is under a full dose.

A so-called pseudo-vagal reaction, sometimes taking place during the inhalation of a vapor, results in a drop in blood pressure very similar to the drop in many of Kratschmer's tracings. None of the writer's blood pressure tracings from healthy rabbits show any initial drop preceding the normal rise. A few rabbits suffering from an upper respiratory infection disclosed an initial drop obviously of vagus origin. In these animals the secretions probably protected the mucous membranes for a time so that the olfactory and trigeminal nerves were not stimulated at first, and consequently inspiration went on normally, resulting in the stimulation of the vagus before these vapors reached the endings of the olfactory and trigeminal nerves.

SUMMARY AND CONCLUSIONS

The inhalation experiments on *normal*, *olfactory* (trigeminals cut) and *trigeminal* (olfactory fibers cut) rabbits and cats confirm the work of others that the inhalation or projection of odoriferous and irritating substances into the nostrils produces marked changes in respiration and circulation. The general effect on respiration is one of depression and inhibition or complete cessation. A strong stimulus may produce a sneeze in a trigeminal rabbit or in a non-anesthetized olfactory rabbit. The circulatory changes consist of a rise in blood pressure and a slowing and strengthening of the pulse.

It is demonstrated by the insufflation experiments in the first subdivision of this report, where similar changes occur in respiration and circulation to those obtained from inhalations of the same vapors, that these changes do not originate from humoral-peripheral or humoral-medulla stimulation as a result of absorption of these vapors, or to secondary humoral or mechanical-vagal stimulation or lack of stimulation due to cessation of respiration.

These respiratory and circulatory reactions may be evoked by stimulation of either the olfactory or the trigeminal endings in the nasal mucosa. In general the reaction is somewhat stronger when both nerves are intact.

Respiratory changes from inhalations. The mild agreeable odors such as extracts of orange, rose, and oil of cloves are the only specific olfactory stimuli found, but at best they cause only a moderate alteration of respiration, and in some rabbits, no change.

Oil of bergamot and anise are usually effective over either the olfactory or the trigeminal. Wintergreen proved to be a fairly powerful stimulant over both nerves.

The disgusting odors have little or no effect on respiration.

Xylol and benzol which are the strongest olfactory stimulants are also powerful trigeminal stimulants, frequently eliciting a sneeze.

Ether, chloroform, formalin, oil of mustard, acetic acid, ammonia, etc., usually classified as trigeminal stimulants, are shown to be almost as effective olfactory stimulants.

Division of the depressors, vagi, and cervical sympathetics at the level of the larynx in no way interferes with the strength of the respiratory reaction in normal, olfactory and trigeminal rabbits.

Circulatory changes from inhalations. Any substance which produces a pronounced or slight alteration of respiration in a normal, olfactory or trigeminal rabbit always causes a correspondingly marked or insignificant change in the circulation.

The alteration of circulation is equally and sometimes more pronounced after severance of the depressor nerves in normal, olfactory and trigeminal rabbits. After double vagotomy the total rise in blood pressure is somewhat reduced; there is also considerable less slowing of the pulse, but the effect on the strength of the pulse is variable.

Inhalation of various vapors results in a rise in blood pressure in the superior vena cava, the same amount of slowing of the atrial pulse as takes place in the carotid pulse, but a conspicuous depression of the atrial pulse waves is evident.

A pseudo-vagal reaction probably caused from an ectopic rhythm is described in connection with certain blood pressure tracings.

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EXPERIMENTAL BONE MARROW REACTIONS

V. The Influence of Water and Alcohol Extracted Liver on Blood Regeneration in Pigeons

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A previous study (1) has shown that in pigeons, with anemia and bone marrow depletion produced by starvation, grain or beef muscle feeding caused a prompt replacement of weight, and a complete rapid regeneration of the blood. In similar pigeons broiled beef liver produced a rapid gain in weight and regeneration of the blood up to a point below normal, followed by a decline of red blood cells and hemoglobin in the peripheral blood, and a definite decrease of the megaloblasts, or the youngest red blood cells in the bone marrow. The bone marrows in the liver fed animals, nevertheless, showed hyperplasia mainly of the cells of the leucocytic series. This is in contrast to the bone marrow of starved pigeons in which the anemia is accompanied by an aplasia of the bone marrow in the radius and the ulna. From theoretical considerations it was suggested that liver was not deficient as a food, but that a substance or substances were present in liver, which inhibited growth of the stem cells of the erythrocytic series.

Numerous investigators have shown by various measures that certain substances inhibit and others stimulate growth. In 1913 Walton (2a) showed that growth of mammalian tissue varies with the medium and that the variation is probably due to the presence of different combinations of inhibiting and stimulating substances. Champy (3) also formulated theories of functional stimuli and inhibiting influences to account for morphological variations in cells. In 1923, Carrel and Ebeling (4a) found antagonistic growth-activating and growth-inhibiting principles in serum, modifying the growth of fibroblasts. The inhibiting action on homologous fibroblasts increased with the age of the animal (4b). It was also established that the growth-activating principle in the serum is thermolabile.

That extracts of organs made with Ringer's solution influence growth of adult mammalian cells in vitro was demonstrated by Walton (2b). The extracts of certain tissues stimulated growth of connective tissue cells to a varying degree, while they either stimulated or inhibited growth of the

parenchymatous organs. Liver alone, of all the tissues tested, inhibited growth both of connective tissue and parenchymatous organ cells.

The inhibitory effect of saline extracts of liver, obtained from adult rabbits and from chick embryos over 12 days old, on growth of mammalian cells in vitro was confirmed by Heaton (5) in the case of fibroblasts obtained from chick embryos. Heaton's work has brought out several facts worthy of attention. He showed that the proliferation of fibroblasts is susceptible to antagonism or inhibition. An inhibiting substance was found in yeast, and was soluble in dilutions of alcohol up to 75 per cent, while a substance promoting growth of fibroblasts was found in embryonic juice. If both are in the same medium, the concentration of the respective substances was found to determine growth or lack of growth. He also discovered that the effectiveness of the inhibiting substance increased with the age of the cultured tissue, lower concentrations being sufficient to prevent growth in spite of the same concentration of growth-promoting substances. Organ extracts tested on in vitro cultures of chicken fibroblasts were found to promote growth to a varying degree, with the exception of saline extracts of the liver in certain concentrations. Liver contains a growth-promoting substance also, which becomes effective in dilute solutions. This growth-promoting substance Heaton found was soluble in 97 per cent alcohol, in which the inhibiting substance was insoluble. An inhibiting substance was detected in other organs also, although in lesser concentrations.

A comprehension of the effect of liver and of liver extracts effective in pernicious anemia on growing cells is of fundamental importance for the understanding of the therapeutic effects of these substances in the disease pernicious anemia. In a former study (1) pigeons were chosen as experimental animals, because it was possible to deplete at least a part of their bone marrow in a physiological manner by simple starvation. During regeneration the red blood cells pass from the endothelium through all the stages of cell division and maturation to the adult red blood cells in a comparatively short time. In addition, the pigeon lends itself admirably to the study of the influences on the megaloblast formation. This is because in contrast to most mammals, megaloblasts in the pigeon apparently form the constant source for the maintenance of an adequate number of red blood cells in the peripheral blood. The predominating cell in the marrow in pernicious anemia during relapse is the megaloblast, and it was thought that some information might be obtained as to the effect of liver on such cells by studying pigeons. It is realized that results obtained with pigeons may not be applicable to man, although fundamental principles governing the growth of cells exhibiting the same function might be assumed to be similar.

The results noted above of the effect of feeding liver to pigeons were

unexpected. The present study thus was undertaken for the purpose of finding out whether liver as such was insufficient as a food for complete blood regeneration in contrast to beef muscle, or whether an inhibitory influence on the growth of primitive red blood cells in vivo was in evidence. The study concerns the effect of extracted liver on the hematopoietic organs of the pigeons and the result may be interpreted to show that liver is not insufficient as a food, but that some unidentified substance or substances are present in liver which suppress megaloblast formation.

Experimental observations. As in the previous experiment (1), pigeons were starved until a definite anemia had been produced; then feeding was begun. The weight of the animals as well as the number of red blood cells and hemoglobin percentage were recorded at frequent intervals. At the end of the experiment, Zenker fixed tissues were studied. The period of starvation was longer in this set of pigeons than in those studied formerly because of the greater vigor of the animals. Many of them were kept on a grain diet for weeks before the starvation was begun. At the end of the starvation period different groups were fed exclusively on beef liver extracted and treated in the following ways:

1. *Water extracted liver.* Beef liver, ground finely in a meat grinder, was extracted with distilled water in the ice box. The first extraction was continued for 24 hours, and the second for 48 hours. The weight of the liver in proportion to the extracting fluid was as 1 to 3 and the reaction was brought to pH 5 by the addition of hydrochloric acid. After extraction the mass was filtered and allowed to drain on the filter paper overnight. The residue was divided and dried in shallow pans. The process of drying was hastened by placing the pans about a foot above a steam radiator. In addition to the above, some liver residue treated in a similar manner from which material containing the active principle effective in pernicious anemia had been separated, was obtained from Dr. Edwin J. Cohn of the Harvard Medical School. This liver residue was thoroughly extracted and consisted mainly of connective tissue and other substances not soluble in water. Animals fed with the water extracted liver constitute group I.

2. *Alcohol extracted liver.* The same procedure was followed as with the water extracted liver except that 45 per cent ethyl alcohol was used as the extracting fluid and the mass was not brought to any definite pH by the addition of hydrochloric acid (group II).

3. *Alcohol treated liver.* The extraction was carried out as described in section 2. The filtrate, however, was distilled under low pressure at 45° to 55°C. The residue obtained after distillation was evaporated in an oven at a temperature of 55°C. and evaporation hastened by passing an air current over the substance. The syrupy mass obtained was incorporated with the liver residue not soluble in alcohol and dried. Thus in this material all the constituents of the liver were present, subjected, however,

to alcohol extraction and, in addition, distillation and evaporation of the filtrate at moderate temperatures. This treated liver was fed to pigeons (group III) to serve as a control so as to determine whether or not any chemical change due to alcohol would influence the results. This material will be referred to as "alcohol treated liver."

As a further control, the previous experiment with broiled liver feeding was repeated on another group of pigeons (group IV). The animals showed the same changes in their blood and bone marrow as previously described (1).

I. *The feeding of water extracted liver.* Nine pigeons formed group I. They were fed with the residue obtained by extracting liver with water. The birds were killed at the end of periods varying from 19 to 34 days. They ate poorly and with the exception noted below were barely kept alive. The average weight of this group at the end of the starvation period was 242 grams. Eight of them continued to lose weight so that at the end of feeding with water extracted liver their average weight was 225 grams. The number of red blood cells at the end of starvation and after feeding averaged 2.6 millions and 2.1 millions per cu. mm. respectively. On the average the hemoglobin fell from 63 per cent after starvation to 51 per cent after feeding the water extracted liver. These data contrast sharply with those for the pigeons fed grain or meat as are set forth graphically in chart 1. It will be noted that in both the grain and meat fed animals there was a rapid gain of weight and a complete regeneration of red blood cells and hemoglobin in about three weeks; while in the broiled liver fed pigeons there was an initial rise with a subsequent fall of erythrocytes and hemoglobin, although a complete restoration of weight occurred. In the pigeons fed on water extracted liver alone, with one exception noted below, the radial bone marrow was aplastic showing the same typical features as seen after a period of starvation. This is in contrast to the bone marrows of animals fed broiled liver which were hyperplastic, with predominating leucocytic hyperplasia. Furthermore, the spleen, liver, and bone marrow showed considerable amounts of golden brown pigment which stained for iron, another characteristic feature at the end of the starvation period.

There was one exception. This animal ate well and gained from 248 to 342 grams in weight over a period of 34 days. The red blood cells, however, remained between 2.8 and 2.5 millions per cu. mm., while the hemoglobin increased so that at the end of the experiment it was 99 per cent. The sections of the femoral bone marrow showed an essentially solid hyperplasia with large red blood cell islands and a great many megaloblasts or young cells of the erythrocytic series. There were perhaps more immature cells than are ordinarily seen in the normal bone marrow. The radial bone marrow was fatty with only slight cellularity at the edges, where there were some very large red blood cell centers with many megalo-

blasts around the edges. The organs contained only small amounts of iron.

Three animals after a period of feeding with water extracted liver during which they lost additional weight, red blood cells and hemoglobin, were fed, in addition to the water extracted liver, Fleischmann's dry yeast, about 1 gram a day. Two of these pigeons were also given about 0.5 gram of a salt mixture, recommended by Osborne and Mendel (6a). The average weight of these three animals just before the yeast was fed was 222 grams, the red blood cells 2.5 millions per cu. mm., and the hemoglobin 70 per cent. Three weeks later the average weight was 295 grams, the red blood cells 3.0 millions per cu. mm., and the hemoglobin 88 per cent. None of these animals regained completely their weight or the number of

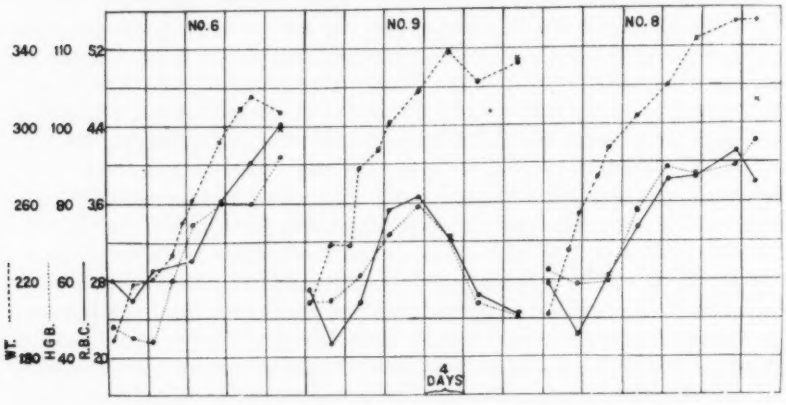

Chart 1. The course taken in the weight, red blood cell count per cubic millimeter and hemoglobin percentage in pigeons fed grain (no. 6) beef liver (no. 9), and beef muscle (no. 8). *X* marks level of weight before starvation was begun.

red blood cells present before starvation was begun. However, the hemoglobin returned to its prestarvation level in the two animals fed both yeast and salts but decreased from 78 per cent to only 75 per cent in the animal fed yeast but no salt mixture. The bone marrows of these three animals were essentially normal. There was more iron in the spleen of the two animals fed the salt mixture than in the one fed yeast alone.

From the observations on this group of pigeons one may conclude that usually liver alone after the extraction with water is deficient as a diet, partially because of low consumption and lack of "vitamin B." In one exception, however, there was gain of weight and increase of the hemoglobin percentage to a normal figure, while the red blood cells remained at the starvation level. Thus a hyperchrome anemia was obtained with an

increase of the megaloblasts in the bone marrow. Yeast, due probably to its content of "vitamin B," increased the appetite and consequently the weight, while the addition of a standard salt mixture also, produced a complete regeneration of the hemoglobin, while the red blood cells remained somewhat below normal in number. The fact that "vitamin B" and many salts necessary for physiological well-being are water-soluble, explains partially the deficiencies of water extracted liver as a food.

II. *The feeding of alcohol extracted liver.* In contrast to the group of pigeons fed the residue from water extracted liver, a second group of ten pigeons (group II), fed with a residue of liver which had been extracted

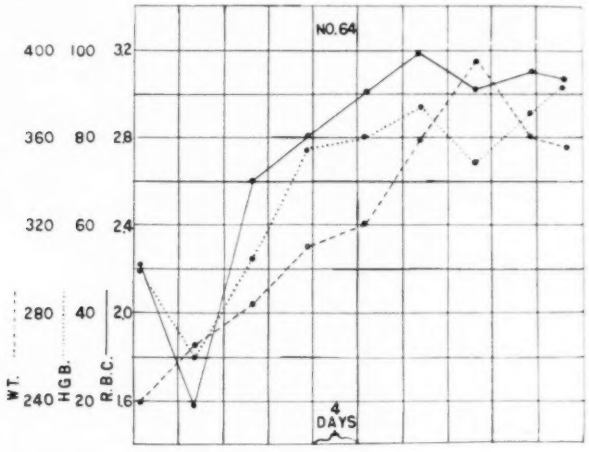

Chart 2. The course taken in the weight, red blood cell count per cubic millimeter, and hemoglobin percentage of a pigeon fed beef liver after extraction with 45 per cent ethyl alcohol. There is no decline of red blood cells and hemoglobin as seen in pigeons fed broiled liver (chart 1, no. 9).

with 45 per cent alcohol, regained their weight, red blood cells, and hemoglobin in a normal manner. Data covering one of these pigeons are presented in chart 2. The average weight of these animals at the end of the period of starvation was 220 grams, and after from 20 to 22 days of feeding 318 grams. The average number of red blood cells during feeding rose from 2.4 to 3.3 millions per cu. mm., and the hemoglobin rose on the average from 70 to 94 per cent. At the end of a similar period of time the pigeons fed broiled liver (chart 1) showed an anemia equal in severity to the one found after starvation (1). Approximately the same values as above were obtained in this group at the end of the period of observation, which varied from 27 to 53 days. Consequently these observations tend to show that

liver, after removal of certain substances extracted with 45 per cent alcohol, is adequate as a food both for the replacement of weight, red blood cells, and hemoglobin, and the maintenance of the same, while broiled liver contains some substance or substances that is inimical to both red blood cell and hemoglobin formation. The microscopical picture of the bone marrows of the pigeons fed the residue of liver after

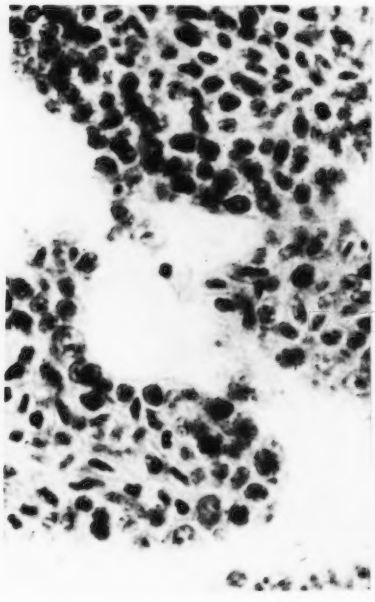

Fig. 1

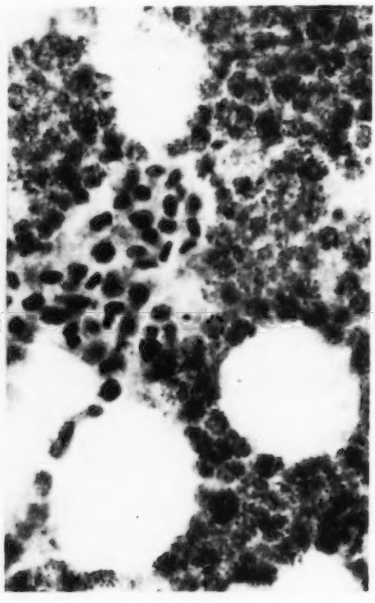

Fig. 2

Fig. 1. Radial bone marrow from a pigeon after 42 days of alcohol extracted liver feeding. The architecture of the bone marrow is normal with large erythrocytic centers and a considerable number of young megaloblasts around the edges. × 750.

Fig. 2. Radial bone marrow, from a pigeon after 24 days of "alcohol treated liver" feeding. The erythrocytic centers are small consisting mainly of mature cells; leucocytic hyperplasia predominates. × 750.

extraction with alcohol showed a normal architecture. The erythrocytic blood centers were moderate in size with a moderate number of megaloblasts or young cells. The bone marrow of one animal showed somewhat more leucocytic hyperplasia than that of the other animals. There appeared to be more hyperplasia of the early cells of the erythrocytic series than in pigeons fed broiled liver, and less erythrocytic hyperplasia than found in those fed grain. The bone marrow of another pigeon fed liver,

extracted with alcohol, showed apparently more erythrocytic hyperplasia than had been encountered in the marrows of normal animals. The liver, of all the pigeons fed broiled liver, showed marked fatty infiltration, while in those fed the residue after alcohol extraction there was little or no fatty infiltration of their livers. It is noteworthy, however, that an animal fed the residue and which showed marked erythrocytic hyperplasia, had considerable fatty infiltration of the liver.

III. *The feeding of alcohol treated liver.* The average initial weight of this third group of six pigeons before starvation was 320 grams, the red blood cells averaged 3.7 millions per cu. mm., and the hemoglobin 115 per cent. At the end of the period of starvation the animals' weight averaged 206 grams, with an average number of red blood cells of 2.6 million per cu. mm., and hemoglobin 87 per cent. The period of feeding varied from 24 to 39 days. At the end of the experiment the average weight was 354 grams, or an average of 34 grams more than the weight before starvation. After feeding the red blood cells averaged 2.7 million per cu. mm. and the hemoglobin 69 per cent. The post-mortem findings of these animals were similar to those of the birds fed broiled liver, except that the bone marrow changes were less marked. On the whole, the entire course taken by the red blood cells and hemoglobin was the same but less striking than with broiled liver. This suggests that in the process of distillation, evaporation, and later mixing, the substances responsible for the changes in the bone marrow and the anemia were weakened or partially destroyed, but were nevertheless present in large enough amounts to give a definite contrast to the results obtained with animals fed alcohol extracted liver. The architecture of representative bone marrows from the pigeons of group II and III is illustrated in figures 1 and 2.

Discussion. In the light of the results obtained in this study, the anemia, with suppression of megaloblast formation in pigeons fed on broiled beef liver, can not be explained on the inadequacy of liver as a food. The hypothesis that some substance, or substances, are present in the liver, which actually suppresses in pigeons blood formation, mainly megaloblast and hemoglobin formation, seems to be the most probable explanation of the phenomena encountered. Perhaps in the liver some substance is present which profoundly affects one group of cells; namely, the so-called "reticulo-endothelial cells." These cells supply megaloblasts in the bone marrow as well as aid to form hemoglobin, primarily in liver, spleen and bone marrow. If one recognizes this and assumes that in the pigeon the "reticulo-endothelial cells" are in constant activity, then a decrease of their functional activity could give a picture identical with the one actually obtained. That substances are present in the liver which inhibit both mammalian and avian cell growth in vitro has been pointed out by Walton (2) and Heaton (5), and such substances may be responsible for the results obtained in feeding liver to pigeons.

That liver diet can affect bodily processes, not related to the hematopoietic function in experimental animals, has been shown by Marine, who found hypertrophy of the thyroid gland in brook trout, dogs (7a), and rats (7b) which had been fed on liver. The hypertrophy of the thyroid may be explained tentatively, perhaps, as a compensatory phenomenon. Robertson and Burnett (8) claim that the hypodermic administration of emulsified liver tissue in rats causes a slight but definite retardation of the Flexner-Jobling carcinoma.

The observations of Osborne and Mendel (6b), Cowgill et al (9) and others, that addition of liver to experimental diets of young rats affords rapid growth of the body in general has been verified in this experimental study with regard to the replacement of weight in starved pigeons, fed exclusively on liver.

If the results obtained in the pigeon represent a fundamental behavior, common to birds and mammals, of a group of cells toward certain substances, the theory previously formulated of "suppression of megaloblast formation," as well as decrease of the functional activity of the entire "reticulo-endothelial system," can logically be applied to pernicious anemia in man if certain facts concerning this disease are kept in mind. The changes in the bone marrow described by Peabody (10) indicate a rapid maturation of the already excessive number of primitive red blood cells in the bone marrow, evidenced by the outpouring of reticulated cells at the time of remission (11 a, b). At the same time, the bone marrow returns from an intensely hyperplastic one with many mitotic figures, a veritable cancerous, or perhaps embryological tissue, to a normal tissue. The increase of the red blood cells and the hemoglobin subsequently is a process without the delivery of many young cells into the circulation. The system of cells present in bone marrow, liver, spleen, and lymph nodes primarily, and grouped for convenience under the name of the "reticulo-endothelial system" apparently performs certain physiological functions in the body. This system probably participates in bile pigment metabolism, hemoglobin formation, blood destruction, and is the source of erythrocytes. A definite train of events might be expected to follow, depending upon whether this system is stimulated, depressed, or performing its function in a normal, balanced manner. Thus if the entire system were overstimulated one could have certain features of pernicious anemia, for example, excessive megaloblast formation, increased blood destruction, increased bilirubin formation, and increased hemoglobin formation. Overproduction of pigments in pernicious anemia was suggested by Whipple in 1922 (12). The decrease of the bilirubinemia at the time of remission, a striking phenomenon, has been pointed out by Broun and associates (13), Minot and Murphy (11c), Russel (14), Ordway and Gorham (15), Dyke and Greener (16), and many other observers. Jungmann (17) from

clinical observations on pernicious anemia suggests that the effect of liver feeding in this disease can be explained by a decrease of the hemolytic function of the "reticulo-endothelial system."

The lack of erythrocytes in the peripheral blood in pernicious anemia is in striking contrast to the enormous increase of megaloblasts or primitive nucleated erythrocytes in the bone marrow, and the excessive deposits of iron containing pigment in the tissues. It is evident that these primitive marrow cells might become functionally adequate by the action upon them of some substance causing their maturation to be hastened. The same results might be brought about in another way, namely, by decreasing the abnormal functional activity of the entire reticulo-endothelial system, thus causing a decrease or cessation of megaloblast formation as well as blood destruction and a decrease of the activity of pigment metabolism. The megaloblasts then would be given an opportunity to mature in a normal manner because the excessively rapid fundamental cell division capable of producing only megaloblasts had been suppressed. Burrows' work with heart muscle cells, commented upon by Uhlenhuth (18) bears on this question:

> We should like to recall the interesting experiments of Burrows in connection with heart muscle cells, as bearing upon the relation between development and differentiation in the cultivated cells. These heart muscle cells can be artificially reduced to a condition at which cell division takes place, at which state they also undergo "dedifferentiation." If, however, the cell division is intercepted the cell is able to develop, and upon reaching the end of its development it begins to pulsate normally, independently of the operation of any specific functional stimuli.

That cessation of cell division is of fundamental importance for the development of the cell to functional activity is realized by Uhlenhuth (18).

> If every cell when about to undergo division renounces its functional structure and becomes embryonal as has often been observed to be the case, ... it is evident that the cells of the culture, even when exposed to functional stimuli, can never take on their original functional structure, as they continually proceed from division to division. It is therefore incorrect to assert that the absence of functional structure is to be ascribed to absence of function. The problematic point is rather the fact that the cells in the cultures never have a chance of developing.

The phenomena observed in the peripheral blood in pernicious anemia at the time of remission have been variously interpreted. It has been suggested from preliminary experiments with extracts of liver that there is an increase in the rate of cell division of the megaloblast (19), and a more rapid maturation of the cells in the bone marrow (10, 11a). There is, however, a fundamental difference between an increased rate of cell division in the megaloblast, and maturation. The latter process probably implies a decrease of cell division.

The question whether the megaloblasts maturate more rapidly because

of a direct effect on them, or whether the already existing megaloblasts in the bone marrow become mature in a normal manner, because new formation and division in the earliest stages are decreased or abolished, is of vital importance. The conception that the liver and certain extracts of liver can furnish directly or indirectly a substance inimical to the existence and proliferation of the megaloblast is supported by this study. In this connection it may be pointed out that the presence of dilute alcohol in the extracting fluid does not prevent the extraction of the principle in the liver effective in pernicious anemia, as has recently been pointed out by West and Nichols (20).

It is also evident that the lack of megaloblasts in the bone marrow of pigeons after liver feeding can not be ascribed to the more rapid maturation of those cells, because with the normal supply of megaloblasts and an increased rate of maturation, one would expect a polycythemia, if increased blood destruction is excluded. The facts obtained can be logically explained, however, on the theory of a suppression of the functional activity of the reticulo-endothelial cells from which the megaloblasts are derived. There would then occur a consequent suppression of the megaloblast formation—a necessary stage in the erythrocytic formation in the pigeon—as well as of hemoglobin formation.

If this theory should prove to be correct, and applicable to man, the discovery of the fact that an active principle in liver is effective in pernicious anemia will have led from a problem of clinical medicine to one of general biology; one of great fundamental importance because of the isolation of a substance for controlling or decreasing growth in vivo of a specific strain of cells in their earliest embryological stages. Maturation of the megaloblast in pernicious anemia would then be a secondary phenomenon. A decrease of the activity of the "reticulo-endothelial system" would appear to explain the experimental results obtained in this study, as well as the phenomena occurring at the time of remission in pernicious anemia.

CONCLUSIONS

1. The effects of feeding water and alcohol extracted beef liver, as well as "alcohol treated liver" and broiled beef liver, on the blood regeneration of pigeons were studied, after the production of a physiological anemia through starvation and consequent bone marrow depletion.

a. Liver residue after water extraction proved to be insufficient as a food for all but one of nine pigeons, both for replacement of lost weight and regeneration of red blood cells and hemoglobin. The bone marrow in the radius and ulna remained aplastic. One animal gained weight, the hemoglobin reached normal values, while the number of red blood cells remained low. The bone marrow showed megaloblastic hyperplasia.

b. The addition of yeast and a salt mixture to the liver residue after water extraction caused an increase in weight, red blood cells and hemoglobin to a point somewhat below normal. Addition of yeast alone proved insufficient for hemoglobin formation. The bone marrows were essentially normal.

c. The feeding of liver residue after extraction with 45 per cent alcohol caused a comparatively normal replacement of weight, red blood cells and hemoglobin, as occurs with any suitable diet for starved pigeons. The microscopical picture of the bone marrow was normal.

d. This is in sharp contrast to pigeons fed broiled liver in which after starvation there was a rapid replacement of weight, an increase of red blood cells and hemoglobin to a point below normal, followed by a definite decrease. This anemia was equal to that after starvation, but was associated with a leucocytic hyperplasia of the bone marrow and a definite suppression of megaloblast formation.

e. The feeding of "alcohol treated liver" gave less marked yet the same kind of results as broiled liver feeding.

2. The information obtained supports the theory that the suppression of red blood cell and hemoglobin formation from liver feeding is due, not to a lack of substances in the liver fed, but to the excess of some inhibitory substance, probably affecting the "reticulo-endothelial system," directly or indirectly, by decreasing its functional activity.

3. It is suggested that if the results obtained in pigeons should prove to be applicable to man, certain phenomena occurring during the remission of pernicious anemia may be explained logically on a decrease of the functional activity of the "reticulo-endothelial system;" especially a decrease of the formation of megaloblasts in the bone marrow. Maturation then would be a secondary phenomenon, the result of decreased cell division.

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THE RESPONSE OF ISOLATED SEGMENTS OF INTESTINE AND UTERUS TO ADRENALIN AT DIFFERENT pH LEVELS OF THE BATH

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It has been shown that the response of the vascular system of the pithed cat to adrenalin varies with the pH of the blood (Burget and Visscher, 1927). This difference in response of the vascular system to adrenalin when the pH of the blood is altered is due to a change in irritability at the site of action of the drug (Burget and Crisler, 1927). These findings apply to the vasoconstrictor mechanism only. The present work was undertaken to determine whether or not the inhibitory innervation to the intestine undergoes similar changes in irritability when the pH of the bath is altered. Many investigators have studied the differences in activity of intestine and uterine segments upon varying the hydrogen ion concentration of the bath in which they were suspended (Evans and Underhill, 1923; Acton and Chopra, 1925; Thienes, 1926) but no attempt has been made to determine the influence of the hydrogen ion concentration of the bath upon the irritability of the inhibitory mechanism of the intestine. Adrenalin was used because it lent itself well for this purpose.

Thirty-five young adult female rabbits were used in this series. The animals were killed at approximately one-thirty o'clock in the afternoon of the day of the experiment by being struck upon the occiput. It was noticed that in every case the animal's stomach was distended with food when the evisceration was made. The diet of the animals consisted of barley and carrots. This diet factor was especially watched for the effect it might have upon the activity of the tissue. Approximately five minutes after the death of the animal the following different methods were used in preparing the tissues: *a*, the tissues to be used were washed carefully inside and outside with 0.9 per cent saline solution and then mounted immediately in the contraction vessel; *b*, mounted immediately without washing; *c*, washed as above, placed for two hours in the refrigerator in a closed vessel at about 10°C. and then mounted; *d*, immediately put in the refrigerator, left there for two hours at 10°C. in a closed vessel, and then mounted without any preliminary washing, and *e*, the unwashed tissues, having been kept for two hours in the refrigerator at 10°C., were washed

with 0.9 per cent saline and then mounted. These preliminary segregations were made in an attempt to explain a "drug action fading phenomenon" to be discussed later in this paper. Tissues over two hours old in most cases proved unsatisfactory for the purposes of this experiment.

Segments of proximal duodenum, proximal jejunum, lower ileum, scybalar colon, and uterus were employed. Gut segments 2 cm. long, and cornate segments of uterus 2 cm. long, were used. Since only one horn of the uterus could be used at a time in each experiment, the other horn was left intact and freely suspended in the bath.

The bath consisted of Locke's mammalian solution, kept at 37.0°C., with a variation of 1.0°C. either way which occurred not faster than 1.0°C. per half-hour. Air was bubbled through from an immersed glass tube of 3 mm. internal diameter at a constant rate of approximately 300 bubbles per minute. The volume of the tissue bath was 250 cc. In order to keep the temperature constant, this bath was surrounded by another bath of 1000 cc. volume, the latter bath being heated by a carefully regulated micro-burner.

Estimations of pH on the bath were made by withdrawing 5.0 cc. with a calibrated pipette, adding 0.25 cc. phenol red solution in alcohol, and comparing colorimetrically to a phenol red set of standards (McCrudden, 1922; Dale and Evans, 1920). This method was found to be accurate enough for the purpose of these experiments. Changes in the pH could be made at will by the addition of N/10 NaOH and N/10 HCl in drop fractions. To allow for a more thorough mixture in the solvent of the acid or alkali, the drug was added about two minutes after the addition of the acid or alkali. pH estimations were made immediately after adding the drug.

Bath pH variations were also effected and studied by exchanging an acid bath for a basic bath, and vice versa, instead of altering the pH of a single bath.

Adrenalin chloride (Parke, Davis & Co.) in 1/1000 solution was used. Fresh dilutions of 1/10,000 were made before each experiment. It was assumed that the chloretone content would be negligible, since this as a factor has been shown by previous investigators to play a small rôle in the action of the drug on the tissues employed. The drug was added to the bath by a drop method from a standard pipette kept for this purpose only. The concentrations of the drug in the bath ranged from 1/8,000,000 to 1/25,000,000 (however, a constant concentration was used through each experiment). The minimal amount that would cause a depression that could be compared quantitatively to a depression caused by a similar dose at a different pH, was employed. Experiments were also done using adrenalin hydrochloride tablets, which were dissolved in 0.9 per cent saline to make a 1/10,000 solution.

The pH of the bath was varied within the limits 6.5 and 8.3, but most of the observations were made within the limits 7.3 to 8.3. It was deemed necessary to vary this much past the physiological limits of blood in order to try the wider pH range which might exist in the living tissues as well as to amplify the gradations in response which might be produced, without any great change in muscle tone.

RESULTS. The results of Evans and Underhill (1923) and Sollmann, von Oettingen and Ishikawa (1928) on the activity of segments of small intestine and uterus when the pH of the bath is altered were confirmed. With segments of small intestine, sudden drops of pH down to 6.5 or even to 6.8 stopped the contractions and produced relaxation, with a permanent loss of tonus, unless the media pH was again raised. A change to the acid side of neutrality caused a decrease in the amplitude of the rhythmical contractions. A sudden change in the opposite direction, e.g., to a pH 8.0 produced an increase of tonus, usually with an increase of amplitude. Sometimes with a high pH value the amplitude of the rhythmical contractions was reduced. Sudden increases of pH caused sudden increases of tonus. Gradual increases of pH caused less alteration in tone but more marked changes in the rhythmical contractions (causing them to increase in rate and amplitude). The rhythmical contractions appeared best at about pH 7.4 and were definitely depressed at pH 6.5; the rate of contraction was decreased in acid and increased in alkaline media. The segments of uterus showed very slight changes in activity due to pH changes from 6.8 to 8.0. The uterus of the rabbit was noticed to be less sensitive to pH changes than that of the guinea pig; when placed in a bath with a constant pH between 7.3 and 7.5 the rabbit uterus exhibted a more constant tonus than the guinea pig uterus. A decrease in pH caused a relaxation of the tonus of the rabbit uterus, with a decreased rate and increased amplitude of the rhythmical contractions.

The duodenum showed in approximately one segment out of every four an atypical response to successive equivalent doses of adrenalin (fig. 1, a). This response was noticed to a lesser extent in the jejunum (one segment out of every six), to a still lesser extent in the ileum than in the jejunum (one segment out of every eight), and least of all intestinal segments in the colon. It occurred in the uterus in about one segment out of every eight. This atypical response manifested itself in that repeated doses of equal amounts of the drug produced successively smaller depressions until, after six to eight doses, no response to the drug could be elicited (even when the dose was doubled or tripled). Care was taken that the tissue had fully recovered from the last dose before another dose was given. This could not be eliminated by changing from the fresh chloretone-drug solution to the tablet solution for a source of the drug, although it seemed to occur more often when using the latter. It also occurred regardless of the

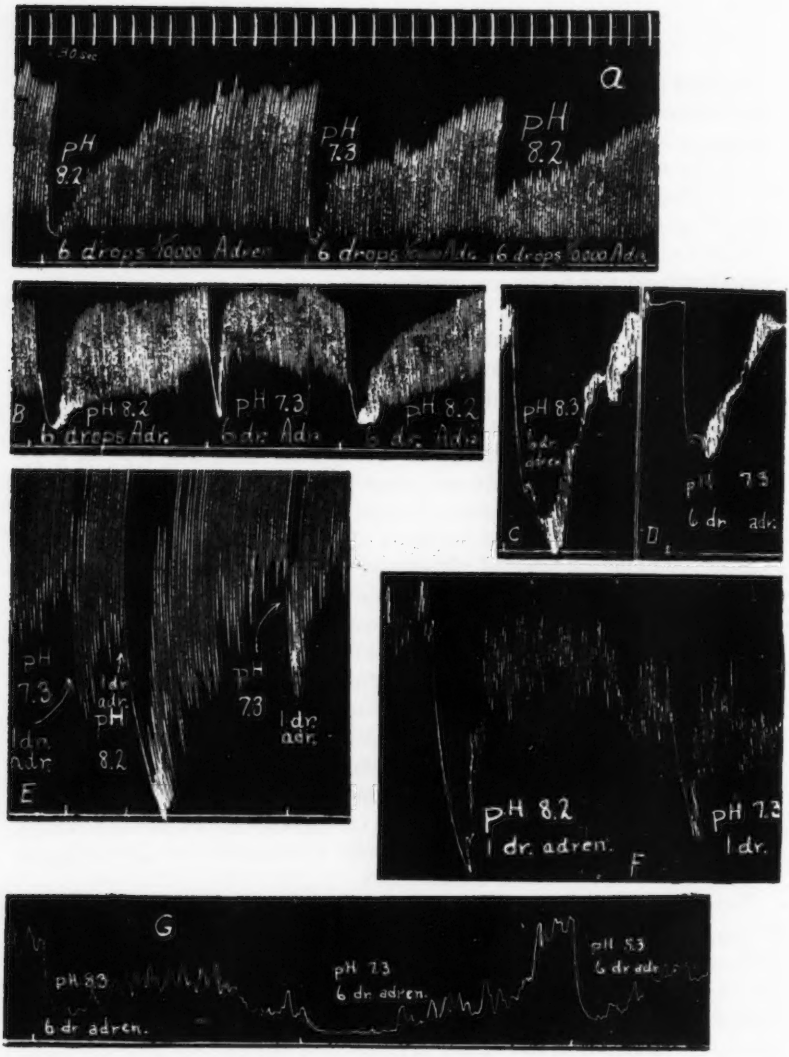

Fig. 1. a. Duodenum. To illustrate the "drug action fading phenomenon" encountered, in that repeated equivalent doses of adrenalin produce successively smaller depressions of tonus and amplitude of contractions.

b, c, d, e. Duodenum. To show the greater and more prolonged depression of tonus and rhythmical contractions in basic than in acid bath as caused by adrenalin, as well as individual differences in gut activity.

f. Ileum. Results similar to those found in duodenum.

g. Colon. Showing that the tonus depression caused by adrenalin is more rapid, and of greater extent but of shorter duration in basic bath and that the depressed rhythmical contractions and tonus recover more slowly at the lower pH level.

methods employed in preparing the segments (see methods above). It was noticed that it could not be eliminated by using Ringer's mammalian solution or Tyrode's solution instead of Locke's solution. A possible diet factor was eliminated because rabbits which showed no such phenomena were receiving the same diet at the same hour as those in which it occurred. No difference in the response according to sex could be seen. Repeatedly renewing the bath or substituting an acid bath for a basic bath would not eliminate it. This phenomenon seemed to be a characteristic of the tissue rather than of the animal, in that, e.g., the duodenum from a certain animal would show it while the uterus from this same animal would fail to do so. In the absence of it the depressions caused by equivalent successive doses on a given segment were equal (i.e., when the pH of the bath was constant, etc.), and could be duplicated again and again until 20 to 30 doses had been given, when the tissues would begin to be sluggish and inactive.

As far as quantitative and qualitative comparisons of the action of adrenalin on the tissues at different pH levels are concerned, only those in which there was an absence of the "drug action fading phenomenon" will be mentioned.

Duodenum. On this tissue equivalent doses of adrenalin produced a greater and more rapid depression of tonus with slower recovery at pH 8.2 than at pH 7.3 (fig. 1, b, c, d, e). Especially was this observed when using tissues which were very sensitive to the drug (e.g., showing a marked depression by 1/25,000,000 adrenalin). The amplitude of the rythmical contractions after a depression returned more slowly to normal at pH 8.2 than at pH 7.3. The rate of the rhythmical contractions was not affected by the drug at any pH. A marked contrast could be seen between the effect of the drug on the tissue at pH 8.2 and at pH 7.3 (fig. 1, e). In a minority of cases equivalent doses of adrenalin in acid bath would cause a depression of tonus to a lower absolute level than in basic bath, but since the tonus in the basic bath was the higher before the drug was added, the total depression in the basic bath was the more marked. Some segments of duodenum showed periodic variations in amplitude much better than others, and a few segments showed no variations at all. The amplitude variations could be augmented by alkalinization and diminished (but not removed) by acidification (within the pH limits used). The less active phase in amplitude in acid media would often be seen as nearly a straight line (fig. 1, c, d). However, such segments showed a more marked initial depression in the basic than in the acid bath (fig. 1, c, d). Such strips were also very erratic in their tonus levels.

Jejunum. With this tissue the results were similar to those mentioned above with the duodenum. In dealing with these segments attempts were made to add the drug during the more active periods in the amplitude cycles. However, it was found that such precautions were not altogether

necessary, since the depressions when effected during the relatively inactive periods in the amplitude cycles seemed to be just as marked as when effected during the active stages. On the other hand it was found that such a precaution is demanded when using uterine segments in order to get consistent results.

Ileum. With segments of ileum, there was a greater and more prolonged depression of tonus, and a longer inhibition of the rhythmical contractions at pH 8.2 than at pH 7.3 (fig. 1, f). The rate of the rhythmical contractions was not affected much in either the acid or basic bath by the addition of the drug. Segments were found which showed no inhibition of rhythmical contractions or depression of tonus at a pH of 7.3 and which with an equivalent dose showed a marked inhibition of rhythmical contractions and depression of tonus at pH 8.2 even though the gut was contracting actively in both cases. A minority of segments of ileum showed an increased tonus on acidification, especially after the drug had been added on previous occasions to the bath.

Colon. Segments of colon were found to show a marked sensitivity to changes in pH as far as tonus was concerned (fig. 1, g). An increase in pH caused a marked increase in tonus while a decrease in pH showed just the reverse. In a minority of segments the tonus remaned fairly constant as long as the air supply, temperature, and pH were not changed, while most of the segments were very variable in their tonus levels. When the segments were removed from the animal and immediately mounted, they would in most cases begin their rhythmical contractions immediately, usually with greater amplitude than they would show if allowed to stand in a refrigerator for 24 hours. Apparently the segments were not absolutely tonically contracted upon immediate removal from the body. However, segments which had been contracting in the bath for 4 to 5 hours or which stood for 24 hours in the refrigerator did have a slightly more relaxed tonus which coincides with the observation of Thienes (1926), probably accounting for our observation that the fresh tissues showed, upon adding the drug, greater drops in tonus than the 24 hour tissues. Evidently the 24 hour tissues (being more relaxed than the fresh ones) are not as capable of further relaxation by the drug as the fresh ones. Equivalent additions of the drug produced a more sudden and greater (since tonus was higher) drop of tonus in the alkaline than in the acid bath (fig. 1, g) However, the lowest absolute level of the depressed tonus was lower in the acid bath even though the fall was not so great. The depressed tonus (i.e., depressed by drug) showed a much slower recovery in the acid media, the effect of the drug apparently being more prolonged at the lower pH level (fig. 1, g). The rate of the rhythmical contractions was not affected by the drug at any pH tried, although the recovery of the depressed rhythmical contractions was faster in the alkaline bath (fig. 1, g). The maximum tonus depressant

action of the drug seemed to occur at about pH 7.2. Evidently the recovery of a tonus (depressed by adrenalin) which is low before the addition of the drug (as in acid media) is slower than when the tonus is high to begin with (as in basic media). The lower absolute limit of tonus depression by equivalent doses in a minor number of segments appeared about the same at pH 7.3 and 8.3. The latent period appeared shorter at a pH of 8.3 than at a pH of 7.3 (fig. 1, g).

Uterus. The uterus showed a nearly constant tonus, regardless of the pH (i.e., between pH levels 7.2 and 8.3). A few segments showed an increase of tonus on decreasing the pH, especially after previous doses of adrenalin had been given. In practically all of the segments employed no difference in the action of the drug could be elicited even in changes of pH from 7.0 to 8.3. A minor number of segments showed a higher stimulatory rise of tonus at pH of 8.2 than at pH 7.3, but in these cases the durations of the tonus changes and amplitude inhibitions were similar. It appears that the pH factor plays a relatively small part here, when one deals with variations between 7.0 and 8.3.

Discussion. It is well known that adrenalin is oxidized more rapidly in the oxygenated alkaline than in the oxygenated acid bath. This might be thought to cause a less prolonged depressant action in alkaline media (especially if we had been using large doses of the drug), but this latter fact was not observed to be the case with the gut segments used. Perhaps this factor does not enter into the physiological response of the tissues. Most of the tissues used in these experiments seemed to withstand excesses of alkali better than excesses of acid (between pH levels 6.8 and 8.3 as lower and upper limits). Possibly the ionized calcium in the bath may play a part in this latter fact and in the variable responses at different pH levels especially if we had employed Tyrode's instead of Locke's solution. It may be added that it is quite essential that one take into consideration the pH of the various tissues in order to more fully appreciate the rôle of pH in affecting the action of adrenalin on the "myoneural junction."

Evidently the inhibitory innervation to the intestine responds to adrenalin at various pH levels with similar differences as does the vasoconstrictor mechanism, while that to the large intestine responds with differences reverse from those of the small intestine to a certain extent. The loss of tonus of the colon at a low pH probably accounts for its slower recovery when the inhibitory mechanism is stimulated in the more acid medium.

SUMMARY

The results of Evans and Underhill (1923) on the activity of segments of small intestine and uterus when the pH of the bath is changed are confirmed. Segments of colon were found to show a marked sensitivity to

changes in pH as far as tonus is concerned. An increase in pH caused a marked rise in tonus while a decrease in pH caused the reverse (between 7.0 and 8.2). The tonus remained constant as long as the air supply, temperature, and pH were unchanged. It was possible to vary the tonus of fresh tissues by drug additon more than of tissue 24 hours old. The segments of uterus showed very slight changes in activity due to changes in pH from 6.8 to 8.3.

Isolated segments of duodenum (more so than those of jejunum, ileum, colon, and uterus, in the order of occurrence) show in about one case out of every four an irregularity in response to adrenalin in that successive doses produce progressively diminishing responses even though an equivalent dose of freshly prepared drug is given each time.

In the absence of this drug action fading phenomenon segments of duodenum, jejunum, and ileum show in most cases after equivalent additions of the drug to the bath at a pH of 8.2 an earlier, greater, more rapid, and more prolonged depression of tonus with a slower recovery of the amplitude of the rhythmical contractions than at a pH of 7.3. The rate of the rhythmical contractions is not affected by the drug at any pH between 7.0 and 8.3. An optimum pH for the depressant action of a minimal dose of adrenalin on these tissues is thought to exist at about 7.8.

In the absence of the drug action fading phenomenon, segments of scybalar colon show in most cases after equivalent additions of the drug to the bath at a pH of 8.3 a greater fall of tonus than at a pH of 7.2. The recovery of depressed tonus and depressed rhythmical contractions is more rapid at a pH of 8.3 than at a pH of 7.2. The rate of the rhythmical contractions is not affected by the drug at any pH between 7.0 and 8.3. An optimum pH for the depressant action of a minimal dose of adrenalin on the colon is thought to exist at about 7.3.

These observations are in agreement with the previous findings as regards the vasoconstrictor mechanism.

Segments of non-pregnant rabbit uterus show no difference in response to minimal stimulatory doses of adrenalin at various pH levels ranging from 7.0 to 8.3.

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THE PROPRIOCEPTIVE INNERVATION OF THE OCULAR MUSCLES

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Since Sherrington (1884) and others demonstrated the existence, in the ocular muscles, of organs of the Golgi type it has remained an open question as to whether the paths originating in these receptors run in the branches of the fifth or third cranial nerve. In a previous paper (Byrne, 1924) it was shown that after lesions causing suspension of function in the proprioceptive organs of the external ocular muscles the homolateral constrictor pupillae became hypersensitive to conjunctival instillations of pilocarpine. The present studies show that the proprioceptive paths taking origin in the external ocular muscles pass exclusively by way of the ophthalmic division of the fifth cranial nerve.

METHOD. This consisted of cutting the ophthalmic division and comparing the constrictor sensitization thereby induced with that induced by: *a*, section of the tendons of the ocular muscles; *b*, section of outer canthus; *c*, section of outer canthus plus section of tendons of all the ocular muscles.

The tendons of the ocular muscles were divided sometimes through a conjunctival incision and sometimes after opening the orbit through a Y incision the stem running along the zygoma whilst the limbs embraced the outer canthus as in Anderson's (1905) operation for removal of the ciliary ganglion.

Within the skull the ophthalmic division may be divided as it enters the Gasserian ganglion without injuring the third or sixth nerve, or the tiny bridge of dura mater containing blood vessels which extends from the petrous bone and tentorium to the anterior clinoid process (fig. 1). After exposing the temporo-sphenoidal lobe the blade of a thin sharp scalpel is inserted near the base of the skull at a point a few millimeters anterior to the posterior boundary of the origin of the zygomatic process from the temporal bone and passed coronally mesialwards and slightly caudalwards in the direction of the Gasserian ganglion. At first the scalpel strikes bone and then by edging mesialwards the point is soon felt to strike into the cushion of nerve tissue under a double covering of dura mater. Carefully advancing, until the point is felt to sink somewhat abruptly over the ledge of bone

Abstracted Protocol. Cat XXXI. Male, 9 lbs.

TIME	REMARKS	PUPILS		MEMBRANES	
		Right	Left	Right	Left
a.m.					
	March 17, 1928				
6:50		7.0	7.0	0.5	0.5
6:51	Pilocarpine 0.75 per cent solution 1 drop in each eye				
7:25		2.0	2.0	1.0	1.0
	March 18, 1928				
8:40		8.0	8.0	0.5	0.5
8:41	Ether. Tracheal cannula inserted				
9:05½	Finished removal of right superior cervical ganglion and making equalization dissection on left side				
9:06	Loose ligature placed around each carotid				
9:08		5.0	5.5	5.0	5.0
9:09	Tied ligatures on carotids				
9:10	Trephined skull on both sides: rongeured away wide areas of bone: incised dura freely				
9:20	Cut ophthalmic division of left fifth nerve				
9:20½	Made equalizing cut in right temporosphenoidal lobe				
9:21	Removed ligatures from carotids				
9:22		2.0	2.0	5.0	5.0
9:26	Corneal reflex absent in left eye: present in right eye				
9:31		2.7	2.5	3.0	3.0
9:31½	Pilocarpine 0.75 per cent solution 1 drop in each eye				
9:58	Paradoxical constriction in left pupil (fig. 2)	2.0	0.7	3.5	4.5
10:00		2.5	0.5	4.0	5.0
10:06	Adrenalin chloride 1:1000 m ii intravenously	8.0	4.0	0.0	0.0
10:10	Atropine sulphate one per cent solution m i intravenously				
10:12	Removed nictitating membranes				
10:20	Paradoxical constriction in left pupil not abolished by atropine	3.8	2.5		
10:30		5.0	2.5		
10:30½	Ergotoxine gr. 2/100 intravenously				
10:34	Ergotoxine gr. 2/100 intravenously				
10:36	Ergotoxine gr. 2/100 intravenously				
10:38	Ergotoxine gr. 2/100 intravenousy				
10:45	Paradoxical constriction in left pupil not abolished by ergotoxine	2.5	1.7		

TIME	REMARKS	PUPILS		MEMBRANES	
		Right	Left	Right	Left
a.m.					
10:46	Corneal reflex absent in left eye: present in right eye				
10:47	Stimulated right and left cervical sympathetic: no effect on pupil				
10:50	Killed animal. Autopsy. Perfect cut of ophthalmic division of left fifth nerve: no injury to bridge of dura nor to third or sixth nerve				

on which the nerve lies the blade is pressed caudalwards through the nerve rather than drawn across it.

When the ophthalmic division has been completely divided the corneal reflex is permanently abolished and stimulation of the cervical sympathetic with the coil at 10.0 cm. fails to dilate the pupil although a slight effect may be noticeable on the membrane. Where the nerve has only been partially divided the corneal reflex becomes restored after a short time. If the third nerve be divided or injured the pupil at once dilates and remains dilated. After section of the nerve an equalizing lesion is made in the opposite temporo-sphenoidal lobe.

To avoid sensitizing the constrictor pupillae through compression (anoxemia) of the constrictor center, or of the third nerve, either of which is possible from intradural arterial hemorrhage, it was found best to do a preliminary decompressive operation on both sides of the skull by freely and symmetrically removing the bone and incising the dura mater.

In cutting the ophthalmic division within the skull the postganglionic fibers of the cervical sympathetic which reach the orbit through this nerve are of necessity severed. The effects of this can be counter-balanced by evulsion of the superior cervical ganglion of the opposite side but as such an operation entails some injury of the afferent vagal branches, which in itself has been shown (Byrne, 1924) to be capable of sensitizing the constrictor pupillae, the method finally adopted was to remove the contralateral superior cervical ganglion and make an equalizing dissection on the homolateral side. In a few instances the vago-sympathetic was cut distal to (above) the superior cervical ganglion and ganglion nodosum. In some instances ergotoxine was used as a means of eliminating the sympathetic mechanisms without injuring the vagal paths.

In some experiments bilateral section of the ophthalmic division was done and the problem of equalizing the effects of injury of the postganglionic sympathetic paths did not arise. Curara was used to show the proprioceptive origin of the paths in the ophthalmic division whose

Abstracted Protocol. Cat 50. Young adult; male

TIME	REMARKS	PUPILS		MEMBRANES	
		Right	Left	Right	Left
a.m.					
	May 6, 1928				
8:04		5.0	5.0	2.0	2.0
8:04½	Pilocarpine 0.75 per cent solution 1 drop in each eye				
8:30		3.0	3.0	1.5	1.5
11:45		3.0	3.0	1.5	1.5
11:45½	Ether				
11:56		4.0	4.0	2.0	2.0
p.m.					
12:07	Loose ligature about each carotid: tracheal cannula inserted				
12:24	After opening both orbits through a Y incision as in operation for removal of ciliary ganglion	7.0	7.0	3.0	3.0
12:29	Tied ligatures on carotids				
12:39	Finished cutting ophthalmic division of right and left fifth nerves: released carotids				
12:41	Corneal reflex present in right and left eye				
1:02	Finished re-cutting of ophthalmic division of right and left fifth nerves				
1:03	Corneal reflex absent in right and left eye	3.0	3.2	3.0	3.0
1:10	Finished cutting tendons of muscles of left eye				
1:10½	Stimulation of left cervical sympathetic coil at 10.0 cm.: no effect on pupil; trace of withdrawal of membrane				
1:11	Stimulation of right cervical sympathetic: no effect on pupil: slight withdrawal of membrane				
1:11½	Cut right and left vago-sympathetic				
1:13		7.1	7.0	3.0	2.0
1:13½	Pilocarpine, 1 per cent solution, 1 drop in each eye				
1:27		7.1	7.0	2.5	2.0
1:27½	Pilocarpine, 1 per cent solution, 1 drop in each eye				
1:42		3.0	7.0	3.0	2.5
1:43	Pilocarpine, 1 per cent solution, 1 drop in each eye				
1:58		2.5	2.8	3.0	3.0
2:00	Atropin sulphate solution, one per cent, 1 drop intravenously				

TIME	REMARKS	PUPILS		MEMBRANES	
		Right	Left	Right	Left
p.m.					
2:03	Pupils about equal: figure 5	8.0	8.1	2.0	2.0
2:20	Pupils equal	7.0	7.0	2.5	2.5
2:21	Killed animal. Autopsy: ophthalmic division of right and left fifth nerves cut through: no injury to bridge of dura nor to third or sixth nerve: tendons of all external ocular muscles cut through in left eye				

interruption caused constrictor sensitization. In control experiments the effect of cutting through the temporo-sphenoidal lobe upon constrictor sensitization was studied. Cats only were used under ether anesthesia. All lesions were verified at autopsy.

Conjunctival instillations of 0.75 to 1.00 per cent solutions of pilocarpine were used to demonstrate the constrictor hypersensitization. Intravenous injections of a one per cent solution of atropin sulphate were used to differentiate essential hyperfunction of the constrictor effectors from constriction due to efferent impulses passing over the third nerve. For further details of technique the reader is referred to a previous paper (Byrne, 1924).

EXPERIMENTAL FACTS. 1. After section of one ophthalmic division the homolateral sensitization was: *a*, slightly greater than that in other eye after section of tendons of ocular muscles (5 animals); *b*, greater than that in other eye after cutting outer canthus (4 animals) (fig. 3); *c*, greater than that in other eye after cutting outer canthus and superior and inferior rectus (2 animals) (fig. 4); *d*, less than that in other eye after cutting outer canthus and tendons of all the ocular muscles (2 animals); *e*, less than that in other eye after cutting third nerve (4 animals). 2. After cutting right and left ophthalmic division, cutting one outer canthus induced further sensitization (2 animals). 3. After removal of right and left superior cervical ganglion and the administration of curara, section of the various divisions of the fifth nerve failed to induce sensitization (3 animals). 4. Lesions of the temporo-sphenoidal lobe such as were made incidentally in cutting the divisions of the fifth nerve failed to induce sensitization (3 animals). 5. Ligation of one carotid for three hours induced homolateral sensitization (3 animals). 6. After bilateral section of the ophthalmic division and opening both orbits, section of tendons of all the orbital muscles of one eye failed to induce further sensitization (4 animals) (fig. 5).

FIG. 1

FIG. 2

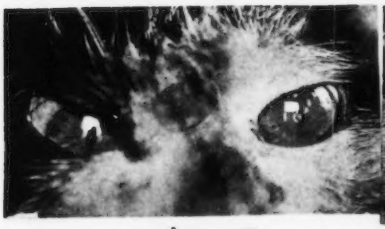

FIG. 3

FIG. 4

FIG. 5

Fig. 1. Shows third nerves on either side of pituitary fossa as they pass forward each mesial to its bridge of dura under which it plunges to join the ophthalmic division as it enters orbit; dark area on left shows where ophthalmic division was cut.

Fig. 2. Paradoxical constriction in left pupil after section of left ophthalmic division: not abolished by atropin or ergotoxine.

Fig. 3. Paradoxical constriction greater in left than in right pupil after cutting ophthalmic division of left fifth nerve and right outer canthus. Ergotoxine had been used to eliminate sympathetic effects.

Fig. 4. Paradoxical constriction more marked in left than in right pupil after cutting left ophthalmic division and outer canthus and superior and inferior rectus of right eye.

Fig. 5. After bilateral ophthalmic section and opening of both orbits, section of tendons of muscles of left eye failed to increase the constrictor sensitization.

DISCUSSION. The sensitization induced by cutting the tendons of all the external ocular muscles in one eye though very marked was not as marked as that induced by section of the contralateral ophthalmic division. This shows that the condensation of proprioceptive paths in the ophthalmic division is of greater functional value than the proprioceptives taking origin in the external ocular muscles.

The sensitization induced by cutting one ophthalmic division was greater than that induced in the other eye by cutting the outer canthus. This shows that the proprioceptive paths in the ophthalmic division are of greater functional value than those taking origin in the orbicularis oculi.

After cutting one ophthalmic division the sensitization was greater than that induced by cutting the superior and inferior rectus plus the outer canthus. This shows that the proprioceptive paths in the ophthalmic division are of greater functional value than those taking origin in the orbicularis oculi and in the superior and inferior rectus.

The sensitization obtained by cutting one ophthalmic division was less than that obtained after section of the third nerve. The flow of efferent constrictor impulses is conditioned by proprioceptive impulses from the striped muscles all over the body but more especially from the anti-gravity muscles and the external ocular muscles (Byrne, 1924). Section of the third nerve is, therefore, equivalent to elimination of the proprioceptive impulses from all over the body in so far as they liberate efferent constrictor impulses, and the constrictor sensitization induced by this operation would naturally be greater than that induced by elimination of the proprioceptive impulses derived from any one organ or set of muscles.

The sensitization induced by section of one ophthalmic division was less than that induced by section of the tendons of all the extra-ocular muscles plus the cutting of the outer canthus. This shows that the combined functional value of the proprioceptive paths taking origin in the extra-ocular muscles and orbicularis oculi is greater than that of the proprioceptive paths running in the ophthalmic division.

After bilateral section of the ophthalmic division cutting one outer canthus induced moderate sensitization. This taken in conjunction with fact proved in paragraph 2 above, shows that not all proprioceptive paths springing from the orbicularis oculi pass by way of the ophthalmic division. Some of them evidently reach the constrictor center by other paths presumably by those running in the middle division of the fifth nerve.

After curara, section of the ophthalmic division failed to induce constrictor sensitization. This shows that the sensitization induced by section of the ophthalmic division was due to interruption of proprioceptive paths (impulses) originating in striped muscles presumably those receiving their sensory nerve supply through the ophthalmic division, viz., the ocular muscles and the muscles about the orbit.

In control experiments lesions of the temporo-sphenoidal lobe resembling, in location and severity, the incidental lesions made in cutting the ophthalmic division, failed to induce constrictor sensitization. This excludes the incidental cerebral injuries as possible factors in the constrictor sensitization induced by cutting the ophthalmic division after a preliminary decompressive operation had been done.

After bilateral section of the ophthalmic division, section of the tendons of all the extra-ocular muscles failed to induce further sensitization of the constrictor mechanism. This, taken in conjunction with the other facts, shows that all the proprioceptive springing from the extra-ocular muscles run in the ophthalmic division.

Ligation of one carotid artery for three hours was followed by constrictor sensitization in the homolateral pupil. Apparently the mechanism here consists of prolonged anoxemia of the constrictor center. In the operations for section of the ophthalmic division the occlusion of both carotids, if kept up for over fifteen minutes, invariably caused the pupils to dilate presumably from anoxemia of the constrictor center caused mainly by the carotid occlusion. With the animal in fairly good condition removal of the carotid ligatures was promptly followed by constriction of the pupils. In the anoxemia following ligature of one carotid, the outflow of impulses from the constrictor center was diminished with consequent sensitization of the constrictor effectors.

Judging from the ease with which anoxemia of the constrictor center, from carotid occlusion, induces sensitization of the constrictor effectors, and from the negative results observed after lesions of the temporo-sphenoidal lobe, and in numerous other experimental and clinical brain lesions, it seems that, after lesions of the brain substance, compression anoxemia of the constrictor center rather than pressure upon the oculomotor nerve is the main factor in the mechanism of pupil inequality, the anoxemia acting directly in causing dilatation and indirectly in causing constrictor sensitization (paradoxical constriction). Usually, however, in head injuries there are also accompanying injuries of the afferent nerves not only in the head but in different parts of the body and these also contribute their quota in causing pupil inequality.

Whilst in general there is no latent (incubation) period in paradoxical constriction such as there is in paradoxical dilatation, nevertheless where bilateral lesions are studied in contrast the sensitization induced by the lesion last made may not be evident to its fullest extent for a short time, say from a quarter to half an hour after the operation. The conditions under which the second lesion is made are such that the traumatic shock from the operation causes prolonged dilatation in the homolateral pupil which tends to overshadow for the time being the effects of the constrictor sensitization. In any event the final determination of the degree of sensi-

tization must always be made in such cases after injections of atropin to sever connections with the third nerve and of ergotoxine to equalize as far as possible sympathetic influence.

The mechanism of constrictor sensitization following section of the ophthalmic division of the fifth nerve consists of interruption of the flow of proprioceptive impulses from striped muscles chiefly from the ocular muscles, orbicularis oculi and other muscles about the orbit with consequent diminution of the flow of efferent constrictor impulses normally conditioned by the varying degrees of muscle tension. Compare Byrne (1924).

The condensation of proprioceptive mechanisms in the ocular muscles has such functional importance that it may be regarded as a special sense-organ comparable to the condensation of proprioceptive mechanisms found in the otic labyrinth with which it functions coöperatively in the reflex adjustments incidental to static and dynamic equilibration. Compare the ocular phenomena observed after destruction of one labyrinth, or upon stimulation of the vestibular nerve, or its equivalent (rotations, etc.) in the intact animal.

But the ocular condensation of proprioceptives has another important function whereby it mediates convergence (proprioceptive) constriction of the pupil and adjustments of the lens (accommodation) in the interests of clear vision and accurate localization of objects with reference to each other (spatial orientation). This mechanism has its counterpart in the ossicles of the middle ear by means of which, owing to their arrangement as a system of levers, the amplitude of the vibrations as they occur in the drum membrane is reduced, whilst stimulus efficiency is preserved or increased around the point where the tilted stapes transmits the vibrations to the perilymph. The part of the mechanism which reduces the amplitude of the vibrations has its analogue in the pupil constrictor mechanism which controls the amount of light entering the posterior chamber of the eye whilst the part that preserves or increases the stimulus efficiency of the vibrations where they pass from the stapes to the perilymph has its analogue in the mechanism of accommodation which condenses the quantitatively reduced stimulus upon the retina in the region of the macula lutea.

The condensation of proprioceptive paths found in the ophthalmic division of the fifth nerve and the relation which these bear to the ocular muscles obviates the necessity for postulating afferent paths in the branches of the oculomotor nerve for the mediation of convergence accommodation and constriction.

As the eye is the most important of the projicient receptor organs it is not surprising to find in it two highly specialized, reciprocally innervated mechanisms for the mediation of the avoidance and approach reactions. One of these mechanisms, the dilator, is operated from the afferent side

through the affective system and on the efferent side through the sympathetic whereas the other, the constrictor, is operated from the afferent side through the critical (includes the proprioceptive) system and on the efferent side through the para-sympathetic. The dilator and constrictor mechanisms may, therefore be regarded as functional outgrowths from the avoidance and approach mechanisms respectively, which in the spinal preparation have their fundamental representatives in the reciprocally innervated flexion reflex and extensor thrust, the former operated through the affective system and the latter through the proprioceptive system. The significance of these two coöperative systems in the development of ordered sensation and psychic function has been touched upon in a previous paper (Byrne, 1924).

CONCLUSIONS

1. The proprioceptive nerves from the ocular muscles run exclusively in the ophthalmic division of the fifth cranial nerve.

2. The proprioceptive paths beginning in the orbicularis oculi pass cerebralwards in part via the ophthalmic division of the fifth nerve and in part via other paths presumably running in the middle division of the fifth nerve.

3. The mechanism of constrictor sensitization following section of the ophthalmic division of the fifth nerve consists of diminution of the efferent flow of impulses from the constrictor center consequent upon interruption of the normal afferent flow of proprioceptive impulses originating in the extra-ocular muscles and orbicularis oculi (in part) and in some other muscles about the orbit.

4. Prolonged anoxemia of the constrictor center causes paralytic dilatation of the pupil and later constrictor sensitization.

5. Pressure anoxemia of the constrictor center is the main, if not the exclusive, factor in the mechanism of pupil inequality following lesions confined to the brain substance.

6. The condensation of proprioceptive mechanisms in the extra-ocular muscles (including the orbicularis oculi) may be regarded as a special sense organ which functions: *a*, coöperatively with the otic labyrinthine mechanisms in equilibratory adjustments; *b*, in convergence (proprioceptive) accommodation and constriction.

7. The mechanism of pupil constriction and accommodation has its close counterpart in the chain of ossicles in the middle ear which reduces the amplitude of the vibrations as they occur at the drum-head and at the same time preserves or increases the stimulus efficiency by condensation at the point where the vibrations are transmitted to the perilymph through the tilted stapes.

8. The proprioceptive paths passing in the ophthalmic division from

the extra-ocular muscles obviate the necessity of postulating afferent paths in the oculomotor branches for the mediation of convergence accommodation and constriction.

9. The pupil dilator and constrictor mechanisms may be regarded as highly specialized, reciprocally related functional outgrowths of the avoidance and approach mechanisms respectively, having their fundamental representatives in the flexion reflex and extensor thrust of the spinal animal.

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STUDIES ON ADRENAL INSUFFICIENCY

VIII. The Survival Period of Untreated Adrenalectomised Cats

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Removal of the adrenals in the cat is easier than in any of the other common laboratory animals except the rat. This has not prevented great discrepancies in the results of different investigators as regards the time of survival. Not only the older work but much of the newer presents a mass of conflicting data, which cannot be employed as a standard, for example, in studying the effects of treatment upon adrenal insufficiency. In any case, we believe that an investigator, in order to be sure of his controls, must accumulate them for himself. We have accordingly determined the time of survival and, as far as possible, the period of good health after removal of the second adrenal in about 50 cats, all healthy at the time of the second operation. The results on 46 cats are given in tables 1 and 2. If for special reasons a few cats have been omitted from the tables, they are mentioned in the text, and any reader who thinks they should go into the tables is free to put them in. It will not make any appreciable difference.

Twenty-eight cats in tables 1 and 2 lived 9 days or longer, i.e., more than four-sevenths of the total number. Between a fourth and a third of the animals lived 11 days or longer. A fourth of the 46 lived 13 days or longer. More than one-fifth survived 2 weeks or longer. One-eighth of the animals lived about 20 days or longer. One cat survived $29\frac{3}{4}$ days; one lived $31\frac{1}{2}$ days. The castrated cat which lived over 35 days will be mentioned later. Cats therefore survive longer than dogs after being deprived of the adrenals. The maximum period is considerably greater than that found by us in dogs. It is likely that with a greater number of cats the progression from the shortest to the longest survival periods would be more uniform, the gaps being better filled in, as in the control dogs. Also, of course, the spread between minimum and maximum periods might be increased.

As it is, there are no really wide gaps in the cat series nor any result which seems out of line with the rest. When an observer is in doubt

TABLE 1

Adrenalectomised male cats

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		ADRENAL WEIGHTS		PANCREAS CONGESTION	REMARKS
	First operation	Second operation				Left	Right		
	kgm.	*kgm.*	*days*	*days*	*hours*	*grams*	*grams*		
142-2	3.12	3.5	15	6¾			0.17	+	Old cat. Lower ileum ++
142-5	3.72	3.5	15	9			0.22	+	Alimentary canal +; 2 gastric ulcers
144-3	2.85	3.06	19	19¾				+++	Stomach +, contains blood
144-8	2.4	2.2	19	29¾				+	Weight 17th day, 2.12 kgm.
144-9	3.23	3.11	22	8½				++	Old cat
146-7	3.99	3.74	12	6½		0.21	0.26	+	Kidneys congested
146-9	2.95	2.72	12	10	4	0.12	0.15	+−	
147-0	2.72	2.65	12	10	21	0.20	0.22	+−	Bloody feces in rectum
147-3	4.11	3.69	10	14	3	0.25	0.29	++	Old cat; small intestine +; 2 small ulcers in pylorus
147-6	3.61	4.02	22	13½		0.23	0.25	−	
148-1	3.13	3.23	20	19¾		0.14	0.18	++	Weight 12th day, 2.92 kgm.; 19th day, 2.75 kgm.
148-2	3.11	3.22	20	6½		0.21	0.22	+	Stomach +. Emesis (bile) 5th day
148-3	3.04	2.15	33	21	1	0.17	0.16	+	Lower bowel +; ulcer at pylorus. Weights 2.16 kgm. 16th day; 2.0 kgm. (20th)
148-4	2.02	2.53	32	9¾		0.17	0.21	++	Stomach +; duodenum +. Weight 16th day, 2.37 kgm.
148-8	2.38	2.47	32	6¾		0.21	0.18	++	Stomach contents bloody; two ulcers. Duodenum ++++; lower bowel + (blood)
153-3	3.23	2.74	24	6¾		0.23	0.20	+++	
153-6	2.65	3.1	25	9¾		0.21	0.21	++	Rectum +
153-7	3.12	2.91	25	5	1½	0.24	0.22	+	
156-8	2.73	3.3	23	9¾		0.25	0.18	+	
156-9	2.13	2.15	23	10¾		0.17	0.18	+++	Small intestine ++; large intestine +
157-0	3.39	3.37	23	10	5	0.22	0.18	++	Stomach and intestines +; 2 erosions near pylorus. Accessory removed at 2nd operation

TABLE 1—*Concluded*

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED	ADRENAL WEIGHTS		PANCREAS CONGESTION	REMARKS
	First operation	Second operation			Left	Right		
	kgm.	*kgm.*	*days*	*days hours*	*grams*	*grams*		
157-1	2.35	2.4	23	$5\frac{1}{2}$	0.31	0.22	+	
157-3	3.39	3.57	23	$7\frac{3}{4}$	0.36	0.24	+++	Old cat. Small intestine +
157-4	3.4	3.57	23	9 5	0.27	0.26	+++	Erosion near pylorus; duodenum +−
157-5	2.42	2.57	24	10 2	0.24	0.20	++	Duodenum ++
158-3	3.26	3.23	17	$14\frac{3}{4}$	0.22	0.17	+	Ulcers in pylorus; large intestine ++

In cats 146-7 to 148-8 inclusive the left adrenal was removed first, in all the others last. The + signs in the remarks refer to congestion of the gastro-intestinal tract.

whether one or more of his results are really out of line in a small series, the test is to see if with a larger series the gaps tend to be filled in. None of the excluded animals lived as short a time as the minimum in the tables. All the cats operated on recovered from the second operation. None died in as short a period as 1 to 2 days.

The average survival period for the 26 male cats in table 1 is 11 days, and for the 20 non-pregnant female cats in table 2, it is $10\frac{3}{4}$ days. As noted in connection with the dogs, we do not think the averages of quantities which vary as much as these periods of survival are of much value especially in small groups of experiments. Certainly the conclusion should never be drawn that because the average duration of survival with a given treatment is somewhat greater than in the controls, the treatment must have been efficacious in "substituting" for the adrenal cortex. It is essential that a fair proportion of the treated animals should survive well beyond the maximum of the controls. As control cats live longer than control dogs, treated cats must live longer than treated dogs in order that a positive result may be inferred.

It was not thought worth while to watch the cats through the night, as was done with most of the dogs. When a cat, which had been alive the previous evening, was found dead in the morning the extra fraction of a day was taken as $\frac{3}{4}$. The operations were done from 9 to 10 a.m., so that the animal would have completed half a day when seen late in the evening before death. It is believed that while some of the cats may have lived a few hours less than $\frac{3}{4}$ day, others would live a few hours longer and in a large series of animals the result would come out about even. When the animals were seen dying the exact survival time is, of course, given.

TABLE 2

Adrenalectomised non-pregnant female cats

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		ADRENAL WEIGHTS		PANCREAS CONGESTION	REMARKS
	First operation	Second operation				Left	Right		
	kgm.	*kgm.*	*days*	*days*	*hours*	*grams*	*grams*		
142-3	2.62	2.75	15	9¾				+	
142-4	2.81	2.68	15	6½			0.13	+	
142-6	4.17	3.9	14	8½			0.28	−	Pancreas mottled, white spots, no congestion
142-8	2.53	2.63	15	7½			0.21	+−	Two ulcers in pyloric end of stomach; duodenum +
142-9	2.7	2.68	15	8	3		0.15	+	
144-0	2.48	2.28	23	7¾				+	
144-1	3.23	3.1	46	31½				++	Had 1 kitten a month before 2nd operation
148-5	1.97	2.01	32	7¾		0.15	0.13	+−	Young cat. Three ulcers (pyloric end). One in duodenum
148-6	3.87	3.25	32	14¾		0.22	0.23	++	Ulcer in pyloric end and 1 in duodenum. Weight 16th day, 2.98 kgm., 14th day, 2.89 kgm.
148-9	1.95	1.65	32	13½		0.22	0.12	++	Weight (6th day) 1.36 kgm., 13th day, 1.35 kgm.
152-4	1.92	1.8	9	2¾		0.15	0.16	++	Youngest cat
152-6	3.89	3.78	9	13¾		0.26	0.32	++	Ulcer near pylorus. Large bowel ++++ and bloody contents
152-7	2.73	2.66	9	9¾		0.24	0.19	++	Parturition 5 weeks before 1st operation
152-8	1.72	1.83	25	9		0.23	0.17	++	
152-9	3.11	3.06	25	9¾		0.17	0.18	+−	Young adult. Gastric ulcer; small intestine +
153-0	2.33	2.46	25	8	5½	0.20	0.18	++	Stomach two ulcers. Old cat
153-2	1.72	2.08	25	9¾		0.27	0.26	++	An accessory removed at 1st operation
158-1	2.43	2.5	17	11¾		0.27	0.27	++	Ulcers in stomach. Duodenum +
158-4	2.4	2.57	17	5¾		0.20	0.17	+++	Had litter 2 months before 1st operation
158-5	2.13	2.29	17	19¾		0.22	0.18	++	Had litter 6 weeks before 1st operation

In cats 148-5 to 148-9 inclusive and in 152-8 to 153-2 inclusive the left adrenal was first excised, in the others last.

As in dogs, the period of good health is usually 2 to 3 days shorter than the survival period and does not vary with the duration of survival. But the onset of the terminal symptoms, especially the anorexia, is often not as easy to determine as in dogs owing to the difference in the habits and psychology of the two animals. It is more common among cats than among dogs to find animals which continue to eat up to about a day before

TABLE 3

Adrenalectomised pregnant cats

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED	ADRENAL WEIGHTS		PANCREAS CONGESTION	REMARKS
	First operation	Second operation			Left	Right		
	kgm.	*kgm.*	*days*	*days hours*	*grams*	*grams*		
147-5	3.2	2.94	10	18¾	0.27	0.25	+−	Advanced pregnancy at 2nd operation; 3 kittens day after operation
148-0	1.84	1.9	20	5	0.14	0.15	++	Early pregnancy. Stomach, 3 ulcers near pylorus
152-5	3.6	3.43	9	2½	0.22	0.18	−	Advanced pregnancy. Coma and in labor 2 days after 2nd operation
153-1	2.89	2.92	25	8 4	0.18	0.21	++	About term. Stomach, 2 ulcers
153-5	2.35	2.53	7	4½	0.22	0.25	++	Advanced pregnancy (2 embryos). Stomach. 5 large ulcers
155-9	2.65	3.05	19	8¾	0.17	0.15	++	Four fetuses (about 50 mm.) born 4th day after 2nd operation. Ulcer in stomach
156-0	2.6	2.46	19	9 4	0.22	0.25	++	One fetus born 2nd day after 2nd operation; no more

Left adrenal removed first in cats 147–5, 148–0 and 153–1. In the others it was removed last.

death. For this reason it is more common to find at autopsy food in the stomach of cats, although we have occasionally seen this in dogs, and a dog has been seen to eat a full meal the day preceding death. Something depends upon the nature of the food.

In cat 147-2, a male, the left kidney was about 3 times as large as the right which was normal. The capsule of the left kidney was maculated, thick and hard. On

ERRATUM

The first sentence on page 166, No. 1 of this volume (LXXXVIII) should read as follows:

As in dogs, the period of good health is usually 2 to 3 days shorter than the survival period, regardless of the duration of survival.

section, intense stasis affecting equally cortex and medulla was revealed. On the lower pole of the kidney under the capsule was a blood clot with signs of recent hemorrhage. It is not known to what extent this condition was a factor in the relatively short survival ($4\frac{3}{4}$ days). For this reason the cat is not placed in table 1. There was no congestion of the pancreas or gastro-intestinal tract.

Cat 147-9, a male, lived $3\frac{3}{4}$ days. At autopsy blood was found in the pericardium. The origin of this blood is not known, but it was thought best not to include the cat in table 1.

In cat 155-7, a female, at the operation for removal of the first adrenal (right) two small accessory nodules (about 1 and 2 mm. in diameter, respectively) were seen at the site of the gland, with the usual vessels and nerves around them. No adrenal could be found. The accessories could have been taken away but it was desired to see whether they would sustain life. After 15 days the left adrenal was removed. The cat lived 14 days, eating little or nothing from the 7th or 8th day onwards. At autopsy the two accessories were found and were of the same size as before. A small vein came from each. They were shown microscopically to consist of cortical tissue. A fibrosed mass about the size of a normal adrenal was found where the right adrenal should have been. It was attached by the lumbo-adrenal vein to the cava. Another small accessory (identified microscopically) was found on the cava just beneath the liver. The pancreas was considerably congested (++). There was some congestion of the jejunum in scattered patches. The ileum showed moderate hemorrhagic congestion. The large intestine was somewhat congested. The right ovary was almost completely destroyed by cystic degeneration. Because of the presence of accessories, not, however, sufficient to save, or perhaps even to prolong the animal's life, this cat is not included in table 2.

In two of the cats (157-0 and 153-2) included in tables 1 and 2, a small accessory was seen on the adrenal vein on the left side and removed at the operation. No accessories were found *post mortem* in any of the cats although most careful search was made in every case.

Two cats (147-4 and 153-4) had been castrated, probably long before they were brought to the laboratory. Opinion is not unanimous as to the effect, if any, of castration upon the survival period after adrenalectomy. So far as we can see from an examination of the literature, there is no reason to conclude that there is any definite effect in either direction. But it was considered best to exclude them from table 1. One of the cats (153-4) lived a shorter time ($7\frac{1}{2}$ days) than the majority of the control animals. It was an old animal. It weighed about 4 kgm. At autopsy an abscess was found in the left lung and the right lung was infiltrated. How much, if at all, this contributed to shorten life is unknown. The other cat (147-4) weighed over 4 kgm. It was very fat. It survived longer than any other control cat we have operated upon (35 days 3 hours). Both are excluded from table 1.

As regards the operation and the care of the animals before and after it, everything said in paper I (Rogoff and Stewart, 1926) on the dog holds true. It is even more important to have well heated quarters, for the cat, a small animal, is more easily injured by cold than the dog. This is of course particularly true of cats soon after the operations. We have seen cats die after excision of one adrenal, undoubtedly from this cause, and we have never seen that in dogs. A cat had one adrenal removed. Its condition after the operation was excellent. Two days later, heat was turned off in the building except the rooms in our laboratory which have a separate

supply of steam. It so happened that the room in which the cat was received no heat for more than 24 hours; the weather turned suddenly cold and the next morning the cat was dead. It is scarcely necessary to prove that cold quarters will harmfully affect a small animal recently operated on.

Shortening of the operation and the anesthesia is quite as important as in the dog. As already stated, adrenalectomy is a very easy operation cats. The usual time from the skin incision to closing of the wound is with us about 5 minutes.

Symptomatology. In our published papers (Rogoff and Stewart, 1925, 1926, 1927, 1928) on this problem which, although a single continuous investigation, is reported for convenience in separate installments, we gave a detailed account of the symptoms in dogs, since the animals studied by previous observers died too soon to permit accurate observations. For cats the symptoms are well described by Elliott (1914). Between cats and dogs certain differences exist, e.g., emesis is less common in the cats. A definite onset of anorexia is often less easy to establish. We have therefore not put into our tables the length of the period of good health. Convulsions and hallucinations are less common than in dogs but are seen. Blood in the stools is more rare than in dogs.

Post-mortem appearance. The striking congestion and often hemorrhages in the gastro-intestinal mucosa with blood in the lumen which were noted in the dogs are less common in cats. When present, congestion is usually less intense and less widespread, while blood in the lumen is occasionally found. Feces in the rectum are sometimes blood-stained. Ulcers in the stomach are more common than with dogs. As in the dog, bile is very frequently found in the stomach. The pancreas is nearly always more or less, not seldom, markedly congested. If the congestion of the pancreas appears less intense in many cases, this may be because of the much smaller thickness of the congested tissue, also of course the smaller size of the veins. There is no doubt, however, that qualitatively at least the condition is similar to that in the dog. It may be mentioned that this is also the case in the guinea pig. The presence of this condition in animals dying of adrenal insufficiency may not only afford another indication of an association between the pancreas and the interrenal tissue, but may be among the contributing causes of death.

Tokumitsu (1923) has suggested the existence of a correlation between the cortex and the pancreas, especially in relation to the metabolism of carbohydrates. However, it is impossible to see how he could have arrived at any conclusion because of the extremely poor operative results, rats dying 4 to 20 hours and rabbits 10 to 20 hours, after removal of the adrenals.

The loss of appetite for fats and interference with their digestion in Addison's disease is suggestive of a correlation between the adrenal cortex

and the pancreas. The aversion to fat is a prominent symptom, appearing as part of, or preceding, the onset of anorexia in adrenalectomised dogs. Adrenal insufficiency is of course more acute in these animals than in human cases where some interrenal tissue is still functioning. Under treatment with extracts the aversion to fats has been seen to become less marked or to disappear in dogs. Amelioration of the symptom (along with other symptoms) in cases of Addison's disease treated during the past two years with extracts (interrenalin) was observed.

It would be a waste of space to quote a great part of the literature as it is without value. Moore and Purinton (1900) state that out of 15 cats 2 died after removal of the first adrenal, one of peritonitis, one of general septicemia, and one was killed by chloroform because of a septic condition of the wound. Of the remaining cats from which the glands were removed in two operations, 7 survived as follows: three under 24 hours; one, 24 hours; one, 33 hours; one, 2 days and 4 hours; one, 4 days and 22 hours. The results were thus extremely bad.

Gradinescu (1913) removed the adrenals in one stage from 9 cats. They survived 12, 16, 20, 30, 40, 55, 56, 84 and 96 hours respectively (average 45½ hours). As dogs operated on in 2 stages survived only for an average period of 42 hours, and rabbits operated on in one stage only 7 hours, it is obvious that the technique was very poor.

The much quoted observations of Strehl and Weiss (1901) gave survival periods for cats doubly adrenalectomised in one stage as follows: 15 cats, 15 to 28 hours; 2 cats, 28 to 47 hours. Five cats from which the adrenals were removed in two stages with an interval of a month, lived 30 to 170 hours after the second adrenalectomy. These are poor results, and anyone who trusts to them, or others as poor, as furnishing a control series for testing the effect of treatment on cats is bound to come to erroneous conclusions. The same may be said of the data of these observers on dogs and other animals. The survival periods are much too short; and the technique cannot have been good.

H. A. Stewart (1914), working on the question whether the adrenals are related to cholesterinemia, found that among the rats employed a pregnant animal was the longest survivor after removal of the adrenals. He recognized that rats were quite unsuitable for such experiments on account of the frequency of indefinite survival. He then used cats and rabbits with a few guinea pigs to see whether pregnancy and lactation exercised an influence on the survival period. The observations on rabbits scarcely require mention because out of 10 animals there was only 1 case of indefinite survival (1 year). Lactation at the time of adrenalectomy could of course have had nothing to do with the long survival. Four other lactating or pregnant rabbits survived as follows: one, 1 day; two, 2 days; and one, 6 days. Of the control rabbits, two lived 1 day; one, 6 days; one died (on the table ?). These are extremely poor results for rabbits.

One lactating guinea pig lived 1 day; two, 2 days; one, 5 days; one, 7 days; one died on the table. Only one control guinea pig is mentioned. It lived 3 days. Obviously no conclusion can be drawn from these results even if it was certain that the whole of the adrenals, especially the right, was taken away. In one (lactating) guinea pig which is described as being alive more than 3 months after removal of the second adrenal it is extremely likely that some of the cortex was left behind.

Of the pregnant and lactating cats, one lived half a day; two lived 1 day; five, 2 days; one, 3 days; one, 4 days; one, 6 days; three, 7 days; two, 9 days; two, 10 days; one, 11 days. Two died on the table, and three were killed (reason not stated).

Of the control cats, one lived less than a day; twelve, 1 day; seven, 2 days; one, 2½ days; eight, 3 days; three, 4 days; two, 5 days; one, 6 days; one, 7 days; one, 15 days. One cat died on the table and several were killed for various reasons. In some cases the experiments were complicated by other operations, and injections of lanolin, etc. In both series many of the animals became infected.

These results are not good enough to permit any conclusion to be drawn. In the paper, however, an attempt is made, by eliminating many of the animals for various reasons, to prove that lactation and pregnancy increase the average duration of life in cats. The symptoms also are stated to be "all much delayed and mitigated." We believe that comparing averages of such figures as are given in the two series is fraught with error, particularly, when a more or less arbitrary selection of results is made.

In any case, the conclusion is not borne out by our own observations (table 3). While in dogs we found in pregnant animals and animals in heat clear evidence of a protective influence, it was not so in pregnant cats. It is true the number of pregnant cats was not great, only seven, but yet perhaps sufficient. The results were certainly no better, perhaps somewhat worse than in the controls. We made no observations on the influence of heat in cats. We think it unlikely that there should be any essential difference between dogs and cats in a matter of this kind. We may expect it to be more difficult to demonstrate an influence of pregnancy in cats than in dogs. Pregnancy as such is an additional, often a serious, handicap to animals subjected to an adrenalectomy. Even if the protective effect would eventually come into play, the animal may not chance to live long enough to get the benefit of this. We saw, not seldom, in dogs cases where the pregnant animal could not overcome the additional handicap and the survival period instead of being lengthened was shortened. Premature labor can, undoubtedly, be brought on by the second adrenal operation (more readily in cats than in dogs) and it depends on the condition of the animal, perhaps on the stage of pregnancy, whether it will successfully cope with the added strain or succumb. In spite of this difficulty, we were able to prove in dogs that the conditions under discussion could protect since many of the pregnant animals (and all of those in heat) lived decidedly longer than any of the control dogs. As stated, we do not think that the relatively poor results in the pregnant cat indicate that there is no potential protective influence of the pregnancy changes, but it will be more difficult to demonstrate them. Corey (1927) was also unable to demonstrate a protective influence of pregnancy in cats. In his control animals the onset of anorexia invariably occurs 50 to 58 hours after the removal of the glands. He finds the average life span of untreated operated cats is only 100 to 120 hours (4 to 5 days). In our experience this is too low, but his results are better than those of Zwemer (1927) who states that cats deprived of both adrenals will survive an average of 53 hours, the extremes being 26 and 111 hours (1 and 4½ days).

The greater susceptibility of pregnant cats than of pregnant dogs to the operation itself is well illustrated by cat 157–2, which, of course, is not included in table 3. The animal was in advanced pregnancy when the first (right) adrenal was removed. She died 4½ days later with her left adrenal still intact. Coma developed on the second day. Clearly, the cat did not die from adrenal insufficiency but probably from some complication associated with the pregnancy and brought on by the operation. Observations on the effect of heat in cats on the survival period are desirable, as it is scarcely likely that heat would be in itself a serious handicap.

Elliott (1914) found that of 25 cats from which the glands were removed in two stages, 9 died on the second or third day. Thirteen lived 6 to 10 days; two survived to the 22nd and 23rd day and one was sacrificed in the 9th week. He attributes the longer periods of survival to compensatory hypertrophy of small accessory adrenals. We presume that the last three cats should be omitted for our purpose.

Marine and Baumann (1927) obtained the following survival periods in 18 cats: 2.0, 2.5, 2.8, 3.0, 3.5, 4.0, 4.2, 4.3, 5.0, 5.2, 5.2, 5.5, 5.8, 7.0, 7.0, 7.5, 8.8 and 12 days. Excluding a kitten which lived 22.3 days, they take 5.3 days as the average period of survival. The kitten was excluded, though no accessory was found, because it was "so out of line with others of this series as well as with our experience of several hundred other suprarenalectomised cats."

A grave defect in the work of Hartman, et al. (1928) on "prolongation of life by extracts" is that they have not published satisfactory controls of their own. To select from the literature a series obtained by other workers and then to use them for comparison with their own treatment series is not, in our opinion, a proper procedure, for more than one reason. Our results show that the survival period of normal cats is far greater than appears in any of the series hitherto published. As a matter of fact, out of more than 90 cats treated with extracts by Hartman and his collaborators only 2 lived much beyond the maximum shown in our 46 control untreated cats (tables 1 and 2), and 3 somewhat exceeded our maximum.

In another paper Hartman (1926) published what we should consider very poor results in a series of cats injected with material of various kinds. As some of this material was almost certainly harmless the corresponding observations constitute a sort of control although not intended as such. Seven cats adrenalectomised at one sitting received intramuscular and intravenous injections of adrenalin and survived on the average only 19.7 hours. Of the animals that received extracts of adrenal cortex of various kinds 5 lived an average of 60 hours; two of 45 hours; 9 of 40 hours; one lived 93 hours; 22 (injected with saline extracts of cortex) an average of 146 hours (about 6 days). These results are not nearly as good as Elliott's on untreated cats and very much inferior to our control cats (tables 1 and 2). It is impossible to deduce from them any protective

influence of the extracts used. MacArthur, Dean and Hartman (1927) observed a group of 13 adrenalectomised cats some of them receiving injection of extracts. The group lived 5 to 26 days (average 12.7 days). This constitutes a negative result when compared with our control tables.

SUMMARY

The survival periods of 46 untreated, adrenalectomised cats, (control series) are tabulated. The maximum period of survival of the cats included in the tables is $31\frac{1}{2}$ days. The average survival period of 26 male cats (table 1) is 11 days; of 20 non-pregnant female cats (table 2) $10\frac{3}{4}$ days. This is very much longer than anything hitherto reported.

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FURTHER OBSERVATIONS ON THE NATURE OF HUNGER CONTRACTIONS IN MAN

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The purpose of this experiment was to determine 1, whether the contractions of an empty stomach are similar in nature to the activity during digestion, and 2, whether contractions of the empty intact stomach can be recorded without a balloon, by way of the esophagus.

Many experimenters have shown at least one type of gastric activity during digestion to be peristaltic. In the early stages of digestion the peristaltic waves originate at the transverse band and pass downward over the pylorus During this phase the corpus and fundus serve mainly as a food reservoir. As digestion advances the waves are initiated progressively higher and higher on the corpus of the stomach and finally at or near the cardiac sphincter. It would seem possible that a similar activity might occur in an empty stomach. Rogers and Martin (1926) believe some hunger contractions to be of this nature, and some to be rather a spasm.

A three-balloon system devised by Templeton and Bollens (1928) for a study of colon motility was used in our study of gastric contractions. Simultaneous tracings, by the ordinary water manometer method, from three different portions of the stomach show hunger motility to be peristaltic. During a period of vigorous contractions and intense subjective hunger (fig. 1, *Part A*) the contractions, or waves, are seen to be initiated by a rise in tone registered by the balloon nearest the cardia followed by a more or less definite contraction at this point. Following this activity, but before its completion, there is a less marked rise in tone at the position of the second balloon, terminating in a definite contraction here, greater than that which occurred at the position of the first balloon. Then, before this is complete, there is a vigorous compression of the third balloon, which is in the pyloric region. Unlike the contraction in the upper portion of the stomach, that seen nearest the pylorus is not preceded by a rise in tone. The contraction is sudden and sharp.

The regular 1, 2, 3, 1, 2, 3 progression in the compression of the three balloons was always more apparent on direct observation of the writing points rising and falling in regular succession, than may be apparent at some points of the tracing. This succession may be obscured when a

second hunger wave commences near the cardia before the preceding wave is completed at the pylorus. In such a case, balloons 1 and 3 may be compressed simultaneously, alternating with compression of balloon 2.

The progressing contraction is least in magnitude in the region of the first balloon and greatest in that of the third balloon. There is furthermore a general rise in tone of the entire stomach up to the end of a hunger period. This rise in tone is greatest toward the pylorus and least toward the cardia.

In the type one contractions the time elapsing between the initiation of the wave with a rise in tone at balloon 1, to the completion of the sharp contraction near the pylorus, is one to two minutes. This, in other words, is the time required for such a wave to traverse the stomach. Near the end of the hunger period, as contractions approached type two, this time is decreased.

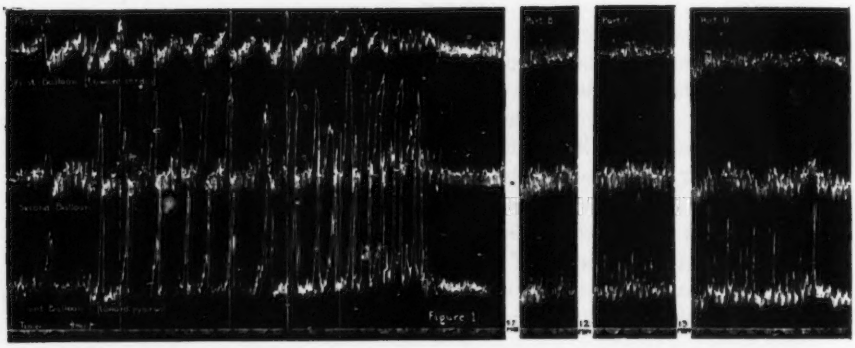

Fig. 1. Simultaneous tracings recording motility from three different portions of the empty human stomach by means of a three balloon system. *Part A:* Conclusion of a vigorous hunger period. *Parts B, C, D:* Commencement and conclusion of a weak hunger period.

In a hunger period of lesser intensity (fig. 1, *Parts B, C,* and *D*) peristalsis may occur in the regions of the second and third balloons only. The activity recorded by the first balloon is negligible, or not apparently correlated with that of the second and third. Peristalsis is initiated by a rise in tone at the second balloon preceding a slight contraction here, while there is little if any rise in tone preceding compression of the third balloon. This would seem to correspond to that phase of gastric activity during digestion in which the peristaltic waves are initiated relatively low in the stomach.

Rogers and Martin (1926) state that hunger contractions are not always associated with peristalsis. Later (Rogers and Martin 1927), continuing these studies by the x-ray method, they asserted hunger contractions to be

of two kinds: 1, "hyper-peristalsis" and 2, circular or tonic contractions of the lower third of the stomach. In our experiment activity was never recorded in the balloon nearest the pylorus except when preceded by contractions at the second balloon, and usually, the first and second balloons. Our observations were made about eight hours after breakfast and showed only type one contractions, sometimes approaching type two at the close of a hunger period.

In the second portion of our experiment we used a modification of the nipple device used by Templeton (1928) on gastrostomy dogs. Into one end of a rubber tube about 7 mm. in diameter were fitted several pieces of glass tubing. These were about 1 cm. long and were placed about 2 mm. apart within the rubber tube. Thus, except for these 2 mm. intervals,

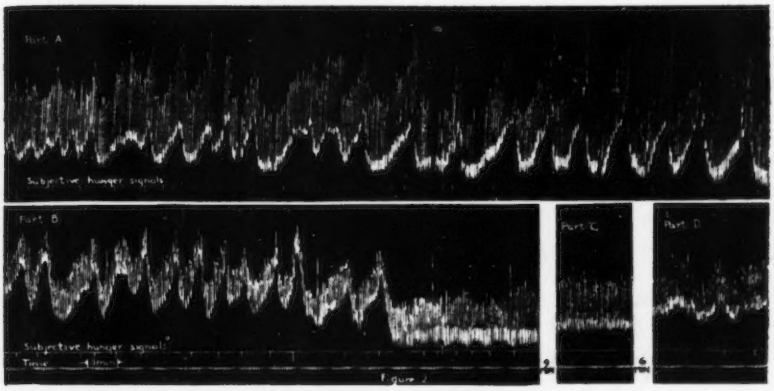

Fig. 2. Gastric motility in the empty human stomach recorded by means of an open tube and nipple, passed down the esophagus. *Part A:* Typical tracing during a hunger period. *Part B:* Conclusion of a hunger period. *Parts C, D:* Rise in gastric tonus during quiescence, and beginning of a new hunger period.

about 20 cm. of the rubber tube was glass lined, yet flexible. This was done so that when a drop of gastric juice got into the open tube it would slide up and down with the contractions more readily than in a plain rubber tube. At the end of the tube so treated, a nipple was fitted.

This was swallowed, or more accurately, pushed down the esophagus until the nipple reached a point just below the diaphragm, as shown by a reversal of the excursions of the manometer writing point when the tube was withdrawn only about 1 cm. Typical tracings were obtained (fig. 2). Each contraction starts as a slow rise in tone and ends in a definite contraction. These variations are superimposed upon a slow general rise in tone, which continues until almost the conclusion of a hunger period. At the end of the hunger period the tone drops more or less suddenly, to

commence immediately another slow rise (fig. 2, *Parts B, C, D*). The general rise in tone progressing over the quiescent period is like that seen on dogs studied through a gastric fistula (Templeton, 1928).

That the contractions recorded are really hunger contractions is apparent from their correspondence to subjective hunger sensations. These were indicated by the subject without his watching the tracing. At the close of the hunger period the subject, however, continued to register his consciousness of hunger (fig. 2, *Part B*). These signals correspond fairly well to feeble tone variations. These may actually have caused the subjective sensation, or the sensation may have been merely a sort of mental resonance.

Observation of gastric hunger motility in dogs by means of the nipple and tube passed down the esophagus is rather difficult. The dog must be lifted to the sitting position and strapped as comfortably as possible. This position seems to affect the character of the stomach movements; at least the appearance of the tracing is unlike that got from a fistula. There is a more gradual closing of the hunger period, without the abrupt drop in tone characteristic of fistula tracings. However, the results on the intact human stomach furnish good additional evidence in support of the view that hunger contractions are not dependent on mechanical stimulation of the gastric mucosa.

SUMMARY

1. Eight hours after breakfast stomach contractions were of type 1, and definitely peristaltic in nature.

2. In a vigorous hunger period nearly the whole stomach is involved in these waves; in a weak hunger period the waves originate nearer the pylorus.

3. Each peristaltic hunger wave is initiated by a slow rise in tone in the cardiac end of the stomach, followed by a definite contraction here; when the wave reaches the pylorus the contraction is sharp and sudden without an initial rise in tone.

4. Activity of the empty stomach in man and dogs can be recorded by the nipple method via the esophagus.

5. In the human, hunger contractions are not dependent upon mechanical stimulation, such as distention or irritation of the mucosa, at least of the pyloric end of the stomach.

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ON ENDOCRINE WEIGHTS IN BROWN LEGHORNS[1]

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From the Whitman Laboratory of Experimental Zoölogy, The University of Chicago

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This report concerns itself with a preliminary investigation of possible sex differences in the weights of the pars anterior of the hypophysis, the thyroids, parathyroids and adrenals of brown leghorn fowls, and with changes occurring in these glands following castration in the male. The weights of the kidneys and the spleen are included but the principal emphasis was laid on the endocrines since, if sex differences could be noted here, these would prove of interest not only in themselves but also as suggesting possibilities for operative experiments.

The investigation is part of the program on the biology of sex now being pursued at the Whitman Laboratory of Experimental Zoölogy under the direction of Prof. F. R. Lillie. We wish to express our appreciation to Professor Lillie for his interest and suggestions and to Dr. L. V. Domm of this laboratory for placing the capons used at our disposal.

MATERIAL. The birds used in this series were all pure bred brown leghorns, obtained from one well-known source and received in the laboratory as one day chicks where they were kept under uniform conditions. The capons were all castrated between 6 and 12 weeks of age. No diseased birds or incomplete capons were included. The observations were begun in the second half of February and continued into the first week of June, 1927. The cocks and hens were all hatched the same day, July 12, 1926, the capons at different dates. Comparable ages and seasons of year at time of dissection are at hand for all three classes of fowls.

The same technique was employed consistently in obtaining the organ weights. The jugulars were severed and the bird suspended head down till bleeding no longer occurred freely; the bird was then weighed. Then the organs in question were dissected out as free from surrounding fat and connective tissue as possible, and placed on filter paper moistened with normal saline in individual weighing bottles immediately following removal. The organs were weighed to one-fifth of a milligram after all had been dissected out. The pars anterior of the hypophysis, the thyroids and

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TABLE 1

Birds in each class arranged in order of increasing age

NUMBER OF BIRD	DATE OF DISSECTION	AGE	WEIGHTS							
			Body	Brain	Hypophysis	Thyroids	Parathyroids	Adrenals	Spleen	Kidney
		days	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
♀645	4-29-27	291	705	3.0714	0.0134	0.1448	0.0142	0.1048	1.1182	5.6820
♀665	4-29-27	291	774	3.0732	0.0218	0.1136	0.0152	0.1428	1.1666	6.4770
♀657	4-29-27	291	889	3.0054	0.0124	0.1070	0.0156	0.1084	1.5062	7.1934
♀647	5- 6-27	298	750	3.1402	0.0102	0.2056	0.0146	0.1324	0.6324	7.3174
♀662	5- 6-27	298	857	2.9610	0.0200	0.1378	0.0216	0.1070	1.3106	7.3056
♀648	5-13-27	305	812	3.1650	0.0152	0.2042	0.0166	0.1586	1.9090	8.1380
♀653	5-13-27	305	839	3.1638	0.0144	0.1024	0.0134	0.0712	1.2110	8.6380
♀666	5-13-27	305	835	3.0330	0.0134	0.1810	0.0384	0.1474	1.7648	9.7140
♀643	5-27-27	319	1,051	3.0174	0.0102	0.1628	0.0206	0.3268	1.1760	10.4326
♀658	5-27-27	319	700	3.1832	0.0102	0.0832	0.0162	0.1052	1.0784	7.8198
♀660	5-27-27	319	818	3.2594	0.0102	0.0972	0.0176	0.1494	1.5872	6.8464
♀654	6- 5-27	328	977	2.9604	0.0112	0.3056	0.0282	0.2016	1.9180	7.9886
♀655	6- 5-27	328	932	3.1720	0.0114	0.2012	0.0178	0.2432	1.2610	9.8948
♂611	3- 4-27	235	826	3.5098	0.0176	0.1096	0.0178	0.2407	1.0362	7.0238
♂694	3- 4-27	235	905	3.2882	0.0168	0.3468	0.0160	0.1936	1.3164	6.6594
♂620	3-11-27	242	1,098	3.4208	0.0106	0.4614	0.0170	0.1428	1.0924	7.9920
♂86B	3-11-27	242	1,154	3.1244	0.0170	0.0708	0.0218	0.1568	1.6912	7.8292
♂606	3-11-27	242	1,192	3.4014	0.0150	0.1692	0.0182	0.2640	1.4440	8.1032
♂609	3-18-27	249	1,362	4.0576	0.0200	0.4930	0.0194	0.2268	1.4666	4.4954
♂619	3-18-27	249	1,212	3.6132	0.0182	0.2642	0.0180	0.2078	1.8764	8.2126
♂601	3-18-27	249	1,214	3.3208	0.0164	0.2996	0.0176	0.1932	1.0316	7.8960
♂603	4-22-27	284	1,247	3.2738	0.0230	0.3874	0.0106	0.2278	1.5818	7.3232
♂630	4-22-27	284	933	3.4084	0.0194	0.1710	0.0174	0.3002	1.0590	5.8950
♂602	5-20-27	312	1,155	3.3638	0.0116	0.2946	0.0092	0.1200	1.6332	8.4312
♂612	5-30-27	322	1,380	3.7388	0.0164	0.1372	0.0150	0.2146	1.2808	8.2126
♂626	5-30-27	322	1,144	3.3928	0.0246	0.1362	0.0096	0.1418	1.2870	7.7228
♂635	5-30-27	322	1,293	3.1410	0.0166	0.6250	0.0156	0.2442	1.8216	8.0984
♂604	6- 5-27	328	1,019	3.4548	0.0156	0.3506	0.0282	0.1748	1.1980	7.3436
♂681	6- 5-27	328	1,212	3.4336	0.0166	0.5410	0.0128	0.2110	1.3630	8.6524
†♂178	2-18-27	239	849	3.2850	0.0248	0.0700	0.0305	0.1912	1.8320	6.5730
†♂124	2-18-27	232	857	3.4284	0.0368	0.1540	0.0182	0.1778	1.2450	8.2184
†♂106	2-25-27	274	1,335	3.3438	0.0196	0.0822	0.0224	0.1466	2.3504	6.6132
†♂85B	2-25-27	228	1,252	3.4940	0.0284	0.1678	0.0138	0.1332	2.5428	10.2634
†♂187	3-25-27	283	723	3.0004	0.0138	0.0326	0.0232	0.0748	0.7026	4.5596
†♂591	3-25-27	276	1,093	3.2406	0.0198	0.0764	0.0418	0.1786	0.9076	6.6412
†♂186	3-25-27	283	1,086	3.0554	0.0162	0.0794	0.0216	0.1410	1.6918	7.3566
†♂80B	6- 8-27	334	1,099	3.5634	0.0226	0.0666	0.0212	0.2312	1.9892	7.6272
†♂583	6- 8-27	320	922	3.1770	0.0130	0.0720	0.0182	0.1514	1.9836	6.6460

♂♂ and ♀♀ hatched 7-12-26.

† ♂ = capon.

parathyroids and the adrenals were then preserved for future histological investigations.

The values obtained in this series of weighings are given in table 1. Here the birds in each class are listed in the chronological order of dissection, that is to say, in order of advancing age and of advancing season of the year. The order of advancing age does not quite hold true for the capons; these were, as stated previously not all hatched the same day.

Discussion. No selection was practised in the collection of the material, its uniformity pertains to origin, age and environment.

The birds in each class may be arranged according to increasing body weight, table not given here, whereupon no correlation appears between body weight and organ weight.

The organs all exhibited a wide range of variability, the order of variability, however, if the spleen and adrenals are omitted, is identical in the three classes of birds. The list of organs beginning with the least variable and proceeding towards the most variable runs as follows: brain, body, kidneys, hypophysis, parathyroids, and thyroids. The adrenals in the hen were found to supersede the thyroids in the degree of variability present, this, however, may be due to the difficulty of clean dissection in the female. In the cock, the adrenals were listed between the kidneys and the hypophysis, and in the capon after the parathyroids; the degree of variability being therefore higher in the castrate. The spleen weights were found to be very consistent in the cock, the range of variability being only slightly higher than that shown by the body weights. In both the hen and the capon the weights of the spleen showed a great diversity. This condition of the spleen in the capon has been noted by Soli (1), and is attributed by him to operative effects.

The individual organs show overlapping in weight from class to class; however, when the average weight of each organ and its average percentage weight per average body weight is obtained, certain sex differences as well as differences subsequent to castration in the male appear at once.

The percentage weights calculated for each organ in each class of bird are alone given below, since these in view of the discrepancy in body weight between cock and hen seem most significant.

	BRAIN	HYPOPHYSIS	THYROIDS	PARATHYROIDS	ADRENALS	SPLEEN	KIDNEYS
♀	0.367	0.00158	0.0203	0.00228	0.0182	0.161	0.946
♂	0.299	0.00150	0.0265	0.00143	0.0177	0.120	0.654
†♂	0.321	0.00211	0.00869	0.00228	0.0154	0.165	0.799

† ♂ = capon.

Sex differences are noticeable in the parathyroids and adrenals, but especially so in the thyroids and hypophysis. In making comparisons

between cocks and hens the average absolute weights and the average percentage per body weight are considered since body weights differ considerably in the sexes, the cock being heavier.

Discussing first the findings in the pars anterior of the hypophysis; the absolute weights in the cock average 0.0172 gram, and in the hen average 0.0133 gram. These figures are not in line with the observed greater weights of the hypophysis in the female albino rat (Hatai, 2), and attributed to a relatively greater development of the pars anterior in the female of this species (Addison and Mary Adams, 3). Expressed as percentages of body weight, the advantage in favor of the cock is practically lost, the percentage weights being 0.00158 gram in the female and 0.00150 gram in the male. It should be noted that all the hens examined were young birds and at the beginning of their first laying season; observations on more mature females might lead to somewhat different results. Still, the weight of the hypophysis of a hen recorded to have a mature egg in the oviduct was only 0.0152 gram, while that of a hen having an immature ovary was 0.0218 gram. From these figures it is difficult to draw any conclusions as to sex differences in the hypophysis of the fowl, though an histological examination to be carried out by one of us may furnish such indications.

However absence of gonad tissue in the male is reflected in an increase in weight of the pars anterior of the hypophysis in the capon. Here the average absolute weight is 0.0216 gram, the percentage per body weight 0.00211 gram, the percentage per brain weight 0.657. In the cock the corresponding figures amount to 0.0172 gram, 0.00150 per cent and 0.500 per cent respectively. The increase in weight of the pituitary after castration has been made the subject of numerous communications; in this connection we may mention the observations of Fichera (4) on a large number of fowls, 50 cocks and 50 capons, the mean weight for the cock hypophysis being 0.0133 gram and for the capon 0.0267 gram. The difference between the two figures is greater than ours, but is in the same general direction. Fichera also reports histological differences, there being a pronounced hyperemia of the capon gland and an increase both in size and number of cells staining with eosin. It is intended by one of us to study this point as well.

In respect to the thyroid, differences were observed in the appearance of the gland in the two sexes examined, and in the capon, even before any weighings were performed. There appeared to be something of a gradation in color and in size, the thyroids of the cock being very prominent and a deep cherry red, those of the hen rather more pink in color but also oval in shape and well set off from the jugulars while in the capon the paired glands were rather flattened in outline in a dorso-ventral direction and their color a pinkish-yellow. The weights obtained in the three classes

seem rather significant. The lower weight of the thyroid in the capon is apparent, as well as the sex difference in favor of the cock; these findings hold true both for the average weight and for the percentage per body weight. A subsequent histological examination of the thyroids included in this series confirmed the sexual dimorphism noted. These observations, which possibly may be associated with the season of year at which the thyroids were collected, will be described in a separate report. The smaller thyroid in the capon is of especial interest and while observations in this laboratory tend to show that the gland is quite susceptible to poor general conditions this cannot be objected in our series, consisting, as mentioned earlier, only of fowls in good condition. The lesser size of the thyroid in Skopecs, a sect practising castration, is mentioned by Lipschutz (5).

The hypertrophy of the hypophysis and diminution in size or retardation in development of the thyroid in the male following castration seems to point toward a relation present between these three organs. Obviously it is impossible to determine from these findings alone whether the changes in the thyroid and hypophysis are both due directly to the elimination of gonadal influence or whether there occurs a primary inhibition of the thyroid with a subsequent enlargement of the hypophysis or vice versa. These questions are to be tested by further experiments.

In conclusion, mention may be made of an asymmetry frequently observed in the location of the thyroids and their relation to the parathyroids. On the left side the thyroid is generally found to be placed rather more anterior to the heart along the jugular and the parathyroid of the same side is often located somewhat caudal to it, perhaps at a distance of about one half to one centimeter. On the right the parathyroid is generally either closely attached to, or else partly imbedded in the posterior pole of the thyroid. The left thyroid also has a tendency towards a somewhat greater development than the right. The parathyroids are in general composed of two spheres slightly different in size forming a figure eight, though we have noted duplications of that number or even more.

SUMMARY

1. A series of weighings was obtained of the adrenals, body, brain, hypophysis (pars anterior), kidneys, parathyroids, thyroids and spleen in brown leghorn hens, cocks and capons.

2. The body weight was found to be similar in the cock and capon and somewhat lower in the hen.

3. The observations recorded are not extended beyond the number and age of the fowls given, nor to other seasons of the year than those at which the weighings were made.

4. The organ weights were found very variable; the thyroid most and the brain least so.

5. The brain weighs most in the hen, less in the capon and least in the cock, in terms of percentage of body weight.

6. In the hypophysis no sex differences could be ascertained on a percentage of weight basis, though caponisation apparently induces an hypertrophy.

7. The thyroids show sex differences (greater weight, darker color) in favor of the cock, the capon thyroid is distinctly the lightest gland in the three classes.

8. There are no pronounced sex differences in the weights of the adrenals; the organ is somewhat lighter as a percentage weight in the capon.

9. The parathyroids are similar in hen and capon and are lighter in the cock.

10. The kidneys weigh most in the hen and are similar in the cock and capon.

11. The spleen weights are similar in hen and capon and lighter in the cock, as percentages.

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THE EFFECT OF MASSAGING THE THYROID ON THE BASAL METABOLIC RATE OF DOGS

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As a corollary to the attempts by Carlson, Hektoen and Schulhof to increase experimentally the hormone output by the thyroid gland and to see if the temporary change in electrical tension in the thyroid gland induced by massage, as reported by Cannon and his co-workers, is in any way related to an increased thyroid hormone output, this work was undertaken at the suggestion of Doctor Carlson. It is an attempt to measure under physiological conditions the changes in basal metabolic rate of trained dogs after massage of the thyroid glands and to follow this basal metabolic rate over a considerable period of time.

METHOD. Six adult dogs were used in these tests, three with apparently normal thyroids and three with enlarged thyroids. All the dogs were given three drops of Lugol's solution each per day to insure enough iodine for hormone production; fed on a constant diet (meat, bread, bone meal) and kept under as nearly the same conditions as possible throughout the course of the experiment. The body weight remained practically constant. The dogs were trained for determining the basal metabolic rate and the method devised by Kunde for using the Benedict-Sanborn respiratory apparatus on dogs was employed. The tests were made at the same time each day.

Massage of the thyroid glands was made by hand manipulation through the skin, the hair on the neck having been carefully clipped away so that it would not interfere with direct massage.

The procedure consisted in taking daily the basal metabolic rate of each dog in the experiment until the normal rate had been determined and then massaging the thyroids vigorously for thirty minutes just after a determination had been made and follow this with another determination thirty minutes later to see if there was any immediate rise in metabolic rate that might be explained as being due to an increased thyroid hormone output as a result of the massage. The metabolic rate was taken each day for several days before another thyroid massage was made.

RESULTS. There was at no time an immediate rise in basal metabolic rate following massage, but on the contrary there was frequently a fall

in the rate; this was probably due to greater relaxation on the part of the dogs for they seemed to enjoy the massage. Frequently the rate was the same before and after the massage.

There was frequently some increase in the basal metabolic rate one, two or three days after massage. This delayed rise was not a constant concomitant of the massage but it occurred so frequently following massage (fig. 2) that it was suggestive that massage might have some positive after effect on the basal metabolic rate. On the other hand, the delayed rise following massage of the thyroid glands frequently did not occur, and in fact there was on a few occasions an actual drop in oxygen consumed. This rise, when it did occur, never exceeded the normal variations that were observed at other times.

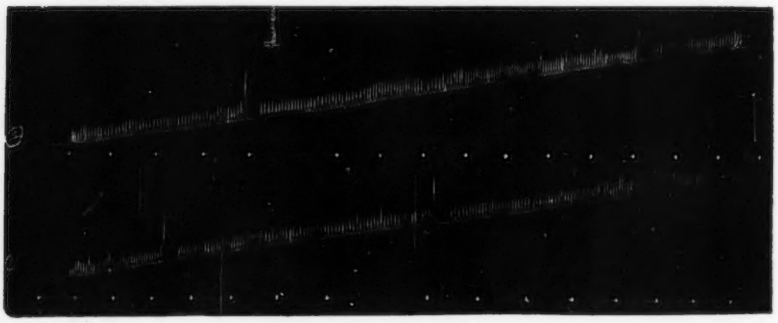

Fig. 1. Dog. Tracing showing rate of oxygen consumption before (1), immediately after (2) thirty minutes massage of the thyroid gland. Time in minutes. In each test the oxygen consumption per minute was 79 cc.

The results on dog 6. This dog seemed in every respect healthy and as for the other dogs, the quantity of food given was adjusted so that the body weight remained practically constant at ten kilograms. The basal metabolic rate of this animal during the latter part of July and the first part of August showed not only marked daily fluctuations but a gradual tendency to increase so that after a month's investigation the oxygen consumption per kilo of this dog averaged about a third above that of the other dogs. By August 16th fair uniformity in basal metabolic rate had been obtained for a number of days consequently a thirty minute vigorous massage of the thyroid gland was made. The following day and subsequently up to September 4th when the experiment was concluded, there was a definite and persistent drop in the basal metabolic rate. We venture no explanation of this unusual occurrence.

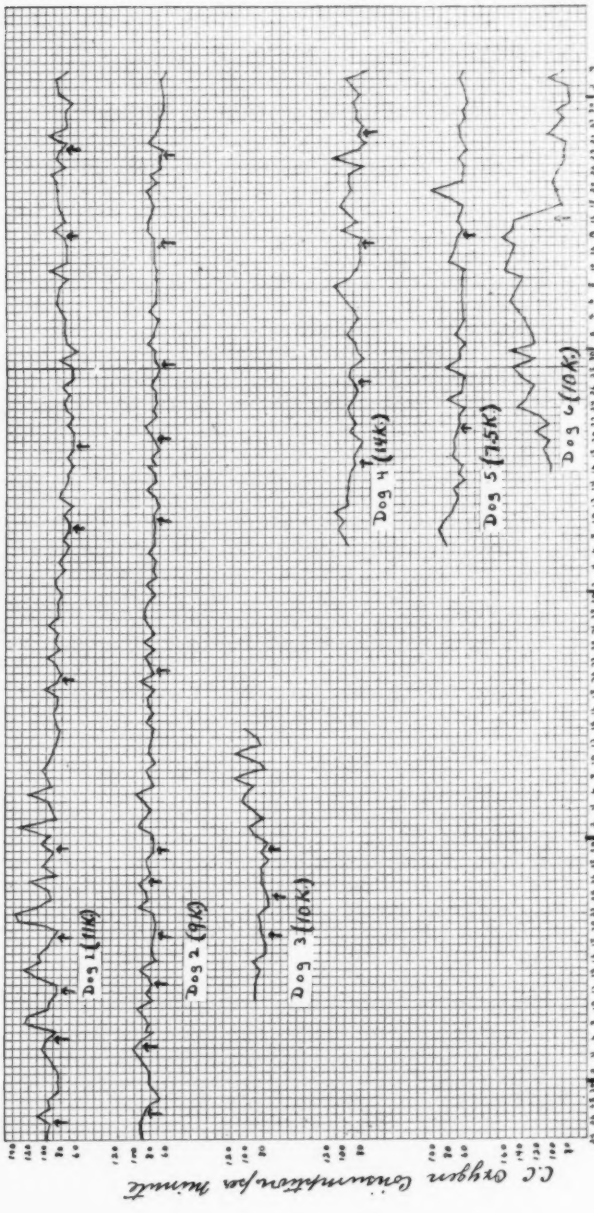

Fig. 2. Chart showing total cubic centimeters of oxygen consumption of each of the 6 dogs during the course of the experiment. In each case the arrows point to the date when 30 minutes' massage of the thyroid gland was made.

SUMMARY

In dogs without anesthesia and under as nearly physiological conditions as possible, there is no immediate and little if any delayed increase in metabolic rate following vigorous massage of the thyroid glands.

We wish to thank Dr. A. J. Carlson for suggesting this experiment and for criticism and advice.

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